MANAGEMENT OF LEAF SPOT DISEASES OF ARECANUT (*Areca catechu* L.)

By

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DECLARATION

I hereby declare that this thesis entitled **" Management of leaf spot diseases of arecanut (*Areca catechu* L.) "** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Dedicated to my beloved parents and my brother

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LIST OF ABBREVIATIONS

%	–	Per cent
°C	–	Degree Celsius
µm	–	Micrometre
@	–	At the rate of
CD	–	Critical difference
et al.	–	And others
Fig.	–	Figure
g	–	Gram
ha	–	Hectare
i.e.	–	That is
ml	–	Millilitre
mm	–	Millimetre
ppm	–	Parts per million
sp.	–	Species (singular)
spp.	–	Species (Plural)
viz.	–	Namely

Introduction

1. INTRODUCTION

Arecanut palm (*Areca catechu* L.) is the source of the common masticatory, popularly known as arecanut, betelnut, supari etc. It is extensively used in India by all sections of the people as masticatory and in several social and religious ceremonies. Arecanut palm occupies a prominent place among the plantation crops in the states of Kerala, Karnataka, Assam, Meghalaya, Tamil Nadu and West Bengal and it is grown in Andra Pradesh, Goa, Maharashtra, Mizoram, Tripura and Andaman and Nicobar Islands (Bavappa, 1982).

Many references were found in several of the ancient Indian literature on arecanut palm. Most important one is "Anjana Charitra" by Sisu Mayana, where references had made to groups of arecanut palms full of inflorescence and branches presenting a nice appearance (Rao, 1982).

In 'Sisupala vadha' by the famous Sanskrit poet Magha, it was mentioned that the soldiers of Sri Krishna from Dwaraka, who on landing in a marshy place, came across a mixed garden of coconut and arecanut and drank coconut water and chewed ripe arecanuts. Similar references were seen in 'Raghuvamsa' by Kalidasa and Amarakosha by Amarsimha Sixth Century A.D (Rao, 1982).

Decondole(1886) in his classical work, "The origin of cultivated palms" stated that the country of origin of arecanut was uncertain and probably Sunda Islands. Beccari (1919) reported Philippines as the origin of arecanut. Arecanut is widely distributed from the Middle East to Far East countries including India, Srilanka, Malaya, Taiwan, Indochina, Sumatra, Indonesia, Philippines, Java, Borneo, Australia, East Indies Islands and Bangladesh (Furtado, 1933; Yama Moto, 1939; Decondole, 1886).

Arecanuts are chewed along with betel leaves, lime, tobacco or spices, which increases the production of saliva and gastric juices, besides it also strengthens the gums and teeth. Its socio-religious uses seen as exchange of betelnuts with betel leaves and coconut, an important part of betrothal ceremonies and marriages. Arecanut has been mentioned for its use as a purgative and in ointment along with several other ingredients for the treatment of nasal ulcers and also reported to be as anthelminthic (Bavappa, 1982)

Its industrial or value added products include scented supari, Gutka, Pan Masala, etc. Areca tannin is being used in leather industry, manufacture of ply boards, textile dye etc. Husk is being used in cardboards, brown wrapping paper, cushions and non-woven fabrics.

Arecanut is one of the most profitable plantation crop grown in India. Incidentally India is the largest producer and consumer of arecanut in the world. In India, arecanut is grown over an area of 3.0 lakh ha and produces 4.0 lakh tonne that accounts for Rs. 3288/- crores to country's GDP (Shetty, 2004). Kerala is the second leading under arecanut cultivation with an area of 99,188 ha and production of 1,16,763 tonnes (Anonymous, 2012). Eighty nine per cent of total area and 86 per cent of total production is accounted by Karnataka, Kerala and Assam.

Arecanut palm is affected by a number of diseases at different stages of growth and development. About 20 diseases, causing varying degrees of damages to the palm have been recorded in India (Bavappa, 1982). Various diseases affecting adult palms are yellow leaf disease, koleroga/ mahali, anabe roga/ foot rot, inflorescence die back and button shedding, bud rot, bacterial leaf stripe, band/ hidimundige, sun scorch(stem breaking), stem bleeding, nut splitting and leaf spot.

Leaf spot and leaf blight diseases are severe on seedlings or younger palms of below 10 years age. They are responsible for destruction of a measurable amount of leaf area and thus lowering the growth rate of the arecanut palm. They were reported to be caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and

Sacc. (Hegde *et al.*,1988) *Pestalotia palmarum* Cooke (Chowdhury, 1946) and *Phyllosticta arecae* Hohnel. (Rao, 1964).

These fungal pathogens are known to cause severe leaf destruction in recent times. However, the detailed work on the leaf spot diseases with regard to their actual damage, etiology and management practices are very scanty. Hence, the present study was undertaken with the following objectives.

- To study the leaf spot diseases affecting young areca palms
- To develop an integrated management practice to contain the diseases.

Review of Literature

2. REVIEW OF LITERATURE

The leaf spot diseases have now become a major problem in arecanut (*Areca catechu* L.). Not much information is available on various aspects of these leaf spots. Hence reviews on coconut and other palms, *Colletotrichum* sp. and *Pestalotiopsis* sp. of different crops have also been presented here.

2.1 History, Symptomatology and Morphology

First report of *Pestalotia palmarum* causing leaf spot disease of arecanut was made by Carl and Bartlett (1922) and it was reported to have spore dimensions of 24-32 µm in length with the mean of 28.3 µm and the length of terminal appendage varied from 19-39 µm with the mean of 25.1 µm.

Single spore culture of *Pestalotia palmarum* from *Borasses flabellifer* L. was reported to be successful in causing leaf spots on *Areca catechu* through wounds (Chowdhury, 1946). He also reported that the conidia measured 14.4 to 21.6 µm by 4.7 to 7.2 µm in nature and 11.7 to 28.3 µm by 3.3 to 6.7 µm in culture and were brown, fusiform, curved, quadri septate, the middle cells dark and those at the extremities colourless, provided with a short hyaline, persistent pedicel at the lower end and three rarely four colourless appendages 7.2 to 25.2 µm long in nature and 4 to 28.3 µm in culture. Another species of *Pestalotia* i.e. *Pestalotia heteronema* was reported to cause grey blight of arecanut (Anonymous, 1950).

Pestalotia palmarum was constantly isolated from the reddish brown discoloured areas on the leaves of arecanut seedlings (Menon et al., 1962). They also reported the withering of affected tissues, production of pustules of the fungus and shredding of entire lamina. These symptoms were severe following heavy rains after severe drought conditions during February to June.

Isolation *of Pestalotia palmarum* from non-sporulating leaf spots of coconut in low rainfall areas was reported by Brown (1975). In Nigeria, large brown leaf spots surrounded by a chlorotic halo and with age the centre of the spots turned grey and shot hole symptoms in coconut, were produced by *Pestalotiopsis palmarum* (Obazze and Ikozun, 1985). They also confirmed the pathogenicity under glass house conditions.

Leaf blight caused by *Pestalotiopsis palmarum* was reported both in seedlings and adult coconut palms in Tamil Nadu (Karthikeyan and Bhaskaran, 1999). He also reported that adult palms of 20 - 40 years of age were highly susceptible to the disease. According to Praveena (1999), the symptoms of grey blight of coconut leaf caused by *P. palmarum* were first seen on the outer whorl in the form of minute yellow specks on the leaflets which gradually enlarged in shape and encircled by a greyish band. The centre of the spot subsequently turned greyish white and the band darkens which in turn was surrounded by a yellowish green halo and later showed blighted or burnt appearance with the production of black acervuli on burnt surface. She also described the morphological features of the fungus as white cottony mycelium, black coloured acervuli and five celled spores with intermediate cells, constricted at dividing septa, 28.37 × 6.86 µm in size, upper end of conidial cells bearing usually three long slender colourless and simple appendages.

Chinara (2012) stated that *Pestalotiopsis palmarum* on coconut leaf were producing small and yellow spots surrounded by a grey margin, developing on older leaves. The centre of these spots later became greyish and spots coalesced, giving the leaves a blighted appearance. On PDA plates the pathogen produced white mycelial growth along with shining black beads of acervuli. The conidia were spindle shaped, five celled, middle cells being deep dark and end cells small, hyaline with 2-4 situlae. Both the apical cells of the conidia were pointed with the two upper median cells darker than the lower median cells.

Phyllosticta arecae causing leaf spot disease of arecanut was reported by Saccardo (1931) and subsequently by Menon (1962) from Palode area of Kerala. Rao (1964) described the pycnidia of *P.arecae* as spherical to globose, dark brown ostiolate, sub erumpent, scattered, few per spot and measured 77.5 to 109.2 µm in dia. Pycniospores were hyaline, single-celled, sub-cylindrical with rounded ends and measured 4.2 to 6.5 µm × 2.75 to 4.0 µm on living leaves of arecanut.

Seshadri et al. (1972) first reported the leaf spot disease caused by *P.arecae* from Karnataka and described that the pycnidia were aggregated, amphigenous, more or less spherical measuring 110-130 µm in dia. Pycnidiospores were minute, filiform, sub-globose to elliptical, hyaline and 10.5 to 12.5 µm × 5.5 to 7.0 µm.

The average per cent infection of *Phyllosticta* leaf spot per leaf ranged from 2.13 to 29.65, 4.18 to 36.70 and 2.50 to 25.35 on arecanut plants of below four years, 4-10 years and above 10 years old respectively. Whereas, the per cent infection per plant was 0.74 to 11.06, 2.24 to 20.72 and 0.58 to 11.85 respectively on the plants of the three groups. The per cent disease index of 4.87 to 28.0 on below four years old plants, 12.62 to 36.38 on 4-10 year old plants and 4.55 to 26.06 on the plants of above 10 years old (Bhat, 1983). He also described the pycnidia of *P.arecae* as more or less spherical, scattered, dark in colour, ostiolate, sub-erumpant and measured 92.57 - 146.02 µm × 88.13 - 135.38 µm. Pycniospores were minute and filiform, hyaline, single celled, guttulate and sub-globose to ellipsoidal with rounded ends. Mucilaginous appendages were present on broader end of the pycniospore. Spores measured 6.34 - 12.88 µm × 5.98 - 9.32 µm. He also reported the maximum growth of the fungus on the 16th day of inoculation with abundant production of pycnidia beyond which the growth was reduced due to autolysis.

Bhat (1988) reported that the average per cent infection per leaf in Uttara Kannada district due to *P .arecae* ranged from 6.18 to 10.63, 10.13 to 15.94 and 7.31 to 10.94 on arecanut of below 4, 4-10 and above 10 years old plants,

respectively, whereas the average per cent infection per plant was 1.98 to 5.78, 4.60 to 9.94 and 2.16 to 4.97 respectively. Similarly, the per cent disease index was 7.17 to 18.04, 13.18 to 24.05 and 7.21 to 15.48 on below 4, 4 - 10 and 10 years old plants respectively.

The blight caused by *P. arecae* was common irrespective of age of the seedlings. The maximum disease index was 16.88, 22.82 and 14.38 per cent on below 4, between 4-10 and above 10 year old palms, respectively in Kumta taluk followed by Honnavar. The percentage of infection per leaf and plant varied considerably from plant to plant in all age groups. The lower most leaf had maximum infection and gradually decreased from older to younger leaves. The top leaves were free from infection in all the age groups indicating the infection of *P. arecae* to the leaves of depleted sugar levels (Bhat et al., 1992).

Hebbar (1992) isolated *P. arecae* from the leaf spot of arecanut leaves collected in North Canara district. The symptoms were initially minute brown specks on leaves, which enlarged to oval to round or irregular spots with dirty ashy brown centre, surrounded by yellow margin. Adjacent spots coalesced to form bigger spots, on which the pycnidia were evident on the ashy brown spotted areas. Severely affected leaves showed blighted appearance, shredding, pre mature drying and finally dropped off. He observed the production of typical, minute, circular brown spots at eight days after inoculation on the unwounded leaves, whereas bigger spots were observed on wounded leaves thus proving the pathogenicity. The pycnidia were scattered, more or less spherical, dark, ostiolate, sub- erumpent, parenchymatous, measured 97.72 - 142.86 µm × 81.26 - 129.94 µm (average 126.21×108.64 µm). Conidiophores were minute and filiform, pycniospores hyaline, one celled, sub globose to ellipsoidal with rounded ends, mucilaginous appendage seen at one end of the pycniospores in some cases, measured 6.79 - 12.42 µm (average 10.21×7.23 µm) and oozed out from the pycnidium in white masses. The survey revealed that all the plants, starting from pre-bearing to prime bearing period were susceptible. Maximum disease index (19.16%) was seen in Honnavar taluk, while least (14.02%) in Yellapur taluk of

Uttara Kannada district. Chennagiri taluk of Shimoga district recorded maximum disease index (17.21%) while minimum (15.70%) in Sagar taluk. The per cent infection per leaf and per plant varied considerably from plant to plant and garden to garden. On potato dextrose agar grey, dark green to green, smooth, wavy to regular margin, having even to uneven surface, moderate to abundant pycnidia and fair to excellent sporulation was observed. Pycnidia were spherical to globose, dark brown, ostiolate, sub-erumpant. Pycniospores oozed out from the pycnidia in white masses. The average size of pycnidia and pycniospores were 121.60×110.90 and 9.20×6.60 µm respectively.

Many types of leaf spots caused by fungi like *Colletotrichum*, *Phyllosticta*, *Helminthosporium* etc. were noticed on arecanut seedlings (Rao and Bavappa, 1961). They also noticed the stunting of severely affected palms.

Dark brown, rectangular to irregular spots on leaves and fronds later leading to defoliation and pitting symptoms on arecanut due to *Colletotrichum gloeosporioides* were described for the first time by Hegde and Hegde (1986). They mentioned that the conidia were hyaline, oblong or cylindrical, non-septate, rounded with oil globules at the centre and measuring 19.14×7.5µm from infected tissue and 17.35×7.17µm from the culture.

In North Canara, a leaf spot disease caused by *C . gloeosporioides* on arecanut was reported by Ramanujam and Chandramohanan (1987) with the incidence ranging from 7.2 to 24 per cent in the gardens surveyed.

Rectanular to irregular, light brown to dark brown lesions on leaf lamina, midrib and basal fronds followed by pre mature defoliation with increased severity was caused by *C . gloeosporioides* (Hegde et al., 1988). The incidence of the disease was observed in a few localized pockets of Sirsi taluk with the maximum incidence in Neernahalli of Sirsi taluk, during 1985. Conidia of the fungus were hyaline, oblong, or cylindrical , non-septate with rounded ends, thin walled having oil golbules at the centre, 14.29 - 24.03×5.34-11.57 µm size with the average of 19.14× 7.51 from the host plant and 12.46-22.85×5.34-11.57µm size with the average of 17.35×7.71 µm from PDA.

Colletotrichum gloeosporioides and *Phyllosticta arecae* were estabilished as causal agents of the leaf spot disease of arecanut in North Canara district as reported by Ramanujam and Chandramohanan (1987). They also reported that the disease index was more during monsoon period (18.5 %) than pre- monsoon period (13.5%). Gardens with average maintenance had higher disease index (14.1 and 19.5 % during pre- monsoon and monsoon, respectively) compared to the well managed gardens (13.0 and 17.6 %). Symptoms included the brown to dark brown or black round leaf spots of various sizes (2-10 mm) with a broad or narrow yellow halo appearing initially and these spots enlarged to 20-30 mm size and later coalesced to form blighted areas in the advanced stages.

The initial symptoms of the leaf spot disease of arecanut caused by *C. gloeosporioides* in Konkan region of Maharashtra appeared as small, irregular, brownish spots surrounded by light yellow halo appearing from base of leaves and forming big necrotic patches after coalescence. In the advanced stage, withering of affected tissues imparting a 'shot hole' effect was recorded as a characteristic symptom followed by premature death of the leaves (Padalkar et al., 1996). Pathogenicity was proved on arecanut and also on African oil palm (*Elaeis gueneensis*), Fish tail palm (*Caryota urens),* Travellers palm (*Ravenalia madogascariensis)* and yellow palm (*Areca lutescens*).

Pradeepkumar (2000) described that the *C. gloeosporioides* causing inflorescence dieback disease of arecanut produced dense, cottony, dirty white to grey aerial mycelium with slightly wavy margin. The colony dia. was about 90 mm with good sporulation and producing acervuli on PDA on 8th day after inoculation. There was production of pinkish droplets of conidial mass in the culture. Microscopic observations revealed the presence of dark setose, disc shaped fruiting body as acervulus with one celled spores, which are hyaline, cylindrical with rounded ends with oil globules at the ends. He recorded the size of the acervulus 120-420 µm with the average of 284 µm and dimensions of the spores were 10.0 to 21.0×5.0 to 10.0 µm with the average of 15.3×7.4 µm.

First authentic proof of *Phomopsis palmicola* (Wint.) Sacc. f. *arecae* Sacc. causing brown, more or less round spots of minute to 4.5cm dia. with darker zonation on arecanut leaves from Assam was reported by Roy (1965) who also described that a few spots coalesced to form bigger irregular greyish blights with full black minute pycnidia. Pycnidia were numerous, amphigenous but mostly epiphyllous, partly immersed in the tissues, ostiolate, brown, pseudo parenchymatous, globose, 107-211μ in dia. conidia hyaline oval to elliptical thin walled, 4.5 - 8.5×2.5-3.4 μm with globules, paraphyses (in culture) hyaline, thread like, bent at the apex, 16-15×1 μm. Colony on PDA slant was white, effuse, fluffy which later turned to chocolate coloured thin stroma on the surface. Fructifications appeared as stromatic mass up to 4 mm high and 2 mm broad, dark coloured, occasionally branched on which the spore mass appeared as creamy white exudates.

Hussain et al. (1992) reported the leaf blight of arecanut caused by *Phomopsis palmicola* in Bangladesh and observed that the best mycelial growth and pycnidial production on PDA medium.

2.2 Pathogenicity test

Tandon et al., (1995) observed that the disease appeared whenever any injured leaf was inoculated. Inoculation of the lower surface of the older uninjured leaves showed symptoms of disease. The older leaves were more easily as well as more severely infected than the younger ones by *Pestalotia mangiferae.* Subsequently Sarkar (1960) observed that when *Pestalotiopsis mangiferae* was inoculated to healthy mature leaves it showed the symptoms of grey blight.

Patel and Patel (1981) observed symptoms on sapota leaf when it was inoculated with *Pestalotia sapotae* which was maintained and multiplied on PDA medium for pathogenicity. Sawant and Raut (1995) reported that necrosis was initiated on leaf lamina within 48 h of inoculation. Symptoms of blight developed easily on mature leaves than on tender leaves.

Ashoka (2003) reported that *Pestalotia palmarum* causing leaf spot disease in arecanut produced the symptoms only when inoculated in the wounded leaves during artificial inoculation, symptoms were observed five days after the inoculation, symptoms appeared as minute circular brown spots later they enlarged in size with ashy brown centre surrounded by brown margin.

Lisa M. Keith et al. (2006) reported that wounding is necessary for artificial inoculation of *Pestalotiopsis* strains on the guava seedlings, he used conidial suspension and symptoms were observed after four to seven days as light brown lesions surrounded by a dark border, which resembled the symptoms occurred in the field.

Sehgal et al. (1965) sprayed *Colletotrichum capsici* spore suspension obtained from 15 days old culture on coriander plants before and after flowering. They noticed the development of symptoms 12 days after inoculation only on the inflorescence, which later extended to the adjacent foliage.

Amusa and Alabi (1996) isolated *Colletotrichum gloeosporioides* from infected pepper (*Capsicum*) and its pathogenicity was confirmed. Ashoka (2003) reported that *Colletotrichum gloeosporioides* produced minute brown lesions four to five days after inoculation with the spore suspension on the wounded leaves, later spots enlarged to typical oval or elliptical spots. Jayalakshmi (2010) reported that *Colletotrichum gloeosporioides* causing anthracnose in pomegranate started showing symptoms from 4th day of artificial inoculation , initially it started as pin head size brown to black coloured water soaked spots, then on the 6th day these spots turned slightly brown surrounded by chlorotic halo with irregular margins.

2.3 Molecular characterization of the leaf spot pathogens

The lack of distinguishable characters between *Colletotrichum* spp. has led to considerable uncertainty regarding the anthracnose fungi (Sreenivasaprasad et al., 1992). Identification and differentiation of *Colletotrichum* species based on morphological characteristics have often been inadequate, as some fungal isolates appeared similar to both *C. gloeosporioides* and *C. acutatum* (Brown et al., 1996). Moreover, the identification of isolates to the species level is difficult and confusing due to the complexity and closely related characters of the species. Molecular analysis of several strains revealed that classification based on morphological data has been erroneous to a great extent resulting in re-classification of several isolates and species (Samuels et al., 1996). In recent years, numerous DNA based methods have been developed for the identification of the fungi. PCR methods are particularly promising because of their simplicity, specificity and sensitivity (Liu et al., 2000).

Internal Transcribed Spacer (ITS) regions located in the rDNA gene complex show extensive sequence diversity among species and is used as signature region for molecular assays, phylogeny and for characterization and identification of the fungi (Ribes et al., 2000). The PCR –RFLP analysis when applied to amplified product of 5.8 S rDNA intervening ITS region has shown promising distinction between different fungal species.

Identification of pathogenic fungi to the species level is essential for initiating early and appropriate disease management strategy. The conventional identification of pathogenic fungi in the laboratory is based on morphological and physiological studies which often requires three or more days and may be inaccurate (Liu et al., 2000; Pryce et al., 2003). Li et al. (2001) have studied the genetic diversity of western gall rust fungus (*Endocronartium harkneii*) collected across western and central Canada from two host pine species. Most of the genetic variation was found between the two host species. Within the host species, variation was among the geographically distant locations than within location.

Joung et al. (2001) identified the phylogenetic relationships among several caterpillar fungi by comparing the sequences of internal transcribed spacer regions (ITS 1 and ITS 2) and 5.8 S ribosomal DNA (rDNA) repeat unit. However, Irina et al. (2005) described that the use of gene sequence analysis alone is not sufficient to identify an isolate at the species level because it has several pitfalls, as it usually involves the submission of sequences to NCBI BLAST and identification of the respective species on the basis of "best hit" or a degree of sequence similarity (e.g., >98 %). This approach is susceptible to errors because: a) deposition of sequences to Gen Bank does not contain a quality control and many records have been submitted without correct species identification; b) some sequences are deposited under the name it has been identified subsequently; c) high correspondence (or not) of a given sequence neither confirms nor refutes species identity unless intra-specific variability of this sequence is known and d) even if it is known that a given species may show nucleotide variation (e.g. 1%), this may not apply for the total sequence area, and nucleotides in some positions may nevertheless be absolutely invariable. Therefore it is required to validate the results of NCBI blast of ITS sequences by considering various parameters.

Rohana et al. (2005) used RAPD analysis for differentiating five different *Colletotrichum* sp. and they observed that RAPD markers could be used in differentiation of individuals better than differentiation of *Colletotrichum* sp. Lisa et al. (2006) reported that use of PCR analysis and amplification of ITS - rDNA fragment can be used for identification and classification *Pestalotiopsis* sp. causing scab disease in Guava.

Sarvottam et al. (2009) observed the genetic diversity among the isolates of *Pestalotiopsis* spp. from different tea growing areas of southern India. He reported that the bands obtained from the RAPD profile were polymorphic in nature and the band size varied from 204 to 3000 bp with an average of 151.7 bands per primer. In case of ISSR, the profile showed that band size varied from 250 to 3212 bp, based on the Jaccard's similarity coefficient matrix he observed that more genetic diversity was observed between the isolates obtained from

different regions than the isolates obtained from the same region of tea growing area.

Bhanwar et al. (2012) reported that molecular characterization as an effective tool for identification of *Pestalotiopsis* sp. causal agent of leaf spot disease in fish tail palm. Meena et al. (2012) used ITS region analysis for identification of *Trichoderma* isolates obtained from different regions and they found variability among the isolates and variability in their nucleotide sequences.

2.4 pH (Hydrogen ion concentration) on growth of pathogens

Pestalotia mangiferae showed maximum growth and sporulation at a pH 5 (Mishra and Chhotaray,1989), whereas Sarkar (1960) reported that *Pestalotia mangiferae* grew well at a pH of 5.5 to 6. Mhaskar and Rao (1981) observed that pH of 5.5 to 6 provided good growth and sporulation of *Pestalotia microspora.*

Sridhara gupta (2000) observed that the *Pestalotiopsis* isolates grew well at pH 6.0 to 7.0 and the growth was decreasing towards alkaline pH 8.0. Younis et al. (2004) reported that the colony growth of *Pestalotia psidii* was maximum at pH 6.5 (90.0 mm) and growth decreased gradually towards alkalinity PH 7.0 (55.0 mm) and pH 8.0 (40.0 mm) as well as acidity pH 5.0 (62.0 mm) and pH 6.0 (70.0 mm) The effect of pH on the growth of *Colletotichum truncatum* was studied by Singh and Shukla (1986) and they noticed the growth on wide range of pH 3.0 to 9.0. The optimum pH range was 5.5 to 7.5 and there was significant reduction in growth of the fungus at pH lower than 5.5 and higher than7.5.

Ekbote (1994) observed that the optimum range of pH for the *C. gloeosporioides* was 5.5 to 7.5. However, maximum growth of the fungus was recorded at 6.6. Sunil Kulkarni (2009) reported that mycelial growth of *C. truncatum* was good at pH 5.5 to 7.0 range.

2.5 Effect of weather factors on leaf spot diseases of arecanut

The intensity of leaf blight (*Pestalotia palmarum*) of coconut was severe on the palms up to 15 years and as the palms grow severity was reduced (Papa Rao and Govinda Rao, 1966).. They also reported that the disease manifests mostly during the cool and humid months and the excessive blight noticed during summer months was only a secondary effect.

Rao et al. (1976) recorded highest number of infections of *P. palmarum* occurred in August, moderate during September and October, again became severe during November. But during December there were very few fresh leaf spots and none at all from January. They presumed that the disease manifests mostly during the cool and humid months and extensive blights during summer is only a secondary effect. Harsh et al. (1987) reported that intensity of *Pestalotiopsis versicolor* was most severe in the late rainy season i.e. September and decreased until leaf fall. Suryachandraselvan et al. (1991) reported that intensity of grey leaf spot on coconut was maximum in December (40.5 %) and minimum in June (23.9 %)). They had mentioned a highly significant negative correlation between disease intensity, relative humidity and rainfall. The number of rainy days has no significant relationship with disease intensity.

An Xianshu and Han Lian Jian (1994) reported that grey leaf spot disease occurred throughout the year and disease incidence increased with rainfall, relative humidity and low atmospheric temperature in August- December. According to them high humidity and monthly mean temperature of 17-24°C were found to be favourable for disease epidemic. High seedling density triggered rapid disease spread and continuous cloud, rainy weather and heavy dew resulted in high disease incidence.

Praveena (1999) reported that grey leaf spot disease of coconut gradually increased from June to January and in subsequent months blighting of leaves was observed and negative correlation was observed between weather parameters and PDI.

Khalequzamman et al. (2003) reported that leaf spot disease of sapota caused by *Pestalotia sapotae* was higher during December (68.85 %) and lowest in July (10.94 %). According to them low temperature, low rainfall and dry weather increases the leaf spot disease.

Chambers (1969) noticed that at least two consecutive days of rain accompanied by cloudiness and high humidity were necessary for infection by *C. truncatum* in bean. Amount of rain was found to be less important than prolonged wetness of the plants by continued intermittent rain, only slight symptoms resulted when shorter periods of rain occurred.

Thakur (1988) reported that the intensity of the mungbean anthracnose has been found negatively correlated with the temperature. The most favourable weather conditions for the disease development were 26 to 30°C temperature, 90 to 100 per cent relative humidity in the morning and 80 to 93 per cent at noon, rains with a wind velocity of about 13 km per hr and the infection of the plants was favoured by overcast and partially cloudy weather but not by clear weather. Thakur and Khare (1991) found that maximum increase in lesion size of green gram anthracnose was recorded when the relative humidity was 100 per cent followed by temperature 27°C and exposure to the light for extended period (72 h).

Ashok Kumar et al. (1999) studied recurrence and development of anthracnose disease (*C. lindemuthianum*) on kidney bean in relation to weather variables in sub-humid mid hill areas (Zone-II) of Himachal Pradesh. The studies revealed that heavy and frequent rains with moderate temperatures (19-25°C) and high relative humidity (>70 %) favoured the progress of disease in terms of vertical and horizontal spread. Correlation and regression analysis of the disease with weather factors further confirmed their role in the disease development.

2.6 *In vitro* evaluation of bio agents

Meena et al (2009) reported that the mycelial growth of *Pestalotiopsis palmarum* was significantly inhibited by *T. harzianum* (68 %) followed by *T. viride* (54 %) and *Pseudomonas fluorescens* (50 %) caused the least inhibition on the pathogen causing post-harvest fruit rot of guava. Saju et al. (2011) found that among different biocontrol agents used for controlling *Pestalotiopsis* sp. infecting large Cardamom *Bacillus subtilis* and *T.viride* showed highest inhibition of 62.6% and 50.9 % in dual culture technique.

Gupta et al. (1991) observed the growth inhibition of *C. lindemuthianum* on french bean by *Gliocladium virens, Trichoderma harzianum* and *T. viride* to 31.66, 44.66 and 88.33 per cent respectively under *in vitro* condition.

Seven *Trichoderma* spp., seven isolates of *Pseudomonas fluorescens*, two isolates of *Bacillus subtilis* and an yeast (*Saccharomyces cerveisiae*) were screened against *C. capsici,* both under *in vitro* and on chilli plants. Among the fungal antagonists, *S. cerevisiae* exhibited the maximum reduction of mycelial growth followed by *T. viride*. Among the bacterial antagonists, *B. subtilis* showed the maximum growth reduction, followed by *P. fluorescens* isolate 27 (Jeyalakshmi et al., 1998).

The effect of antagonist alone and in combination of plant extract and chemicals was studied by Chandrasekaran and Rajappan (2002). In individual *Trichoderma viride* at 0.4 per cent showed 50 and 52 per cent disease index of leaf anthracnose and pod blight respectively in combination with *Lawsonia inermis* at one per cent, alum at 0.1 per cent and *Trichoderma* at 0.4 per cent through seed treatment and foliar spray.

Trichoderma harzianum, T. viride, T. hematum, Gliocladium virens, Bacillus sp. and *Ralstonia fluorescens* were screened for their efficacy against *C. capsici* causing anthracnose of bell pepper. *T. hematum* was found as best biological control agent followed by *T. viride, Bacillus* sp. and *P. fluorescens* (Pathania et al., 2004). Laxman (2006) opined that among fungal bioagents

tested, *T. harzianum* was found to be most effective in *C. truncatum* growth suppression followed by *T. viride*, whereas *Bacillus subtilis* (TNAU) isolate showed maximum mycelial growth suppression among bacterial bioagent.

The combination of *Trichoderma viride* and *T. harzianum* and *Gliocladium virens* were found to be potential antagonists against *Colletotrichum gloeosporioides* causing mango anthracnose (Gud and Raut, 2008).

2.7 *In vitro* evaluation of plant extracts

Leaf, flower, stem and root extracts of *Vinca rosea* L. inhibited spore germination, sporulation and mycelial growth of *Pestalotia* sp. Leaf extracts were more antifungal than the extracts of other parts (Narain and Satapathy, 1978). Pandey et al. (1983) reported that extracts of *Azadirachta indica* Adr. Juss and *Ocimum sanctum* L. inhibited germination of *P. psidii* spores. Leaf extracts of two *Eucalyptus* spp. restricted mycelial growth of *Pestalotiopsis mangiferae* (El - sayed et al., 1986)

Antifungal agents isolated from leaf extract of *Rhaphiolepsis umbellate* L. inhibited *Pestalotia* sp. (Watanabe et al., 1990). Garg and Siddiqui (1992) reported that cumaldehyde isolated from seeds of *Cuminum siminum* L. exhibited antifungal activity against *P. psidii*.

Rai (1996) reported that screening of 17 medicinal plants against *Pestalotiopsis mangiferae* revealed 14 antimycotic whereas three plants *Argemone mexicana* L., *Caesalpinia bonducella* (L.) Roxb and *Cassia fistula* L. accelerated the growth of the pathogen. The maximum antifungal activity was shown by *Eucalyptus globulus* (88 %) and *Catharanthus roseus* (L.) Don (88 %) followed by *Ocimum sanctum* (85.50 %) and *Azadirachta indica* (84.66 %).

Praveena (1999) reported that leaf extracts of *Parthenium* sp., *Ocimum* sp., and *Clerodendron* sp. @ 10 % concentration effectively inhibited the mycelial growth to 99.9, 99, 86.41 % respectively of *Pestalotia palmarum*, the causal agent of grey blight in coconut. Islam et al. (2004) reported that Garlic extract at four and five per cent concentrations inhibited 100 % radial growth of

P. palmarum. The Neem and Tulsi at five per cent inhibited 12.82 and 13.40 % of radial growth respectively. No inhibition was found in doses of onion, marigold, betel vine and ginger plant extracts.

Saju et al. (2011) observed that combined aqueous extract of *Artemesia vulgaris* and *Schima wallichi* at 15 % conc. inhibited *Pestalotiopsis* sp. upto 74.5 %, the pathogen causing leaf streak in Cardamom. Anand et al. (2012) recorded that maximum inhibition of *Pestalotia psidii* causing guava canker was obtained with 44 % Tulsi leaf extract and 15 % Neem leaf extract upto 63.5 and 64.7 % respectively.

Gupta et al. (1981) reported that conidial germination of *C. capsici* was inhibited by Phytonoids of *Allium cepa* L., *Allium sativum* L., *Azardiracta indica* L., *Ocimum basilicum* L. and *Leucas* spp. Further, Shivapuri et al. (1997) noticed that among the plant extracts tested against fungal pathogen *C. capsici*, *Azadirachta indica*, *Datura stramonium* L., *Oscimum sanctum* L., *P. longifolia* and *Vinca rosea* L. were found more fungitoxic.The extracts of Ginger, Garlic and Neem gave excellent control of seed borne *C. truncatum* when soybean seeds were dipped for 30 min. in these extracts (Hossain et al.,1999). Garlic (60.13 %), Neem (57.14 %) and Eucalyptus oil (61.93 %) were found most promising botanicals against *C. truncatum*, which showed higher inhibition of mycelial growth at 10% conc. (Laxman, 2006).

Prasanna Kumar et al. (2006) evaluated three plant extracts against *Colletotrichum gloeosporioides*, among these Ocimum leaf extract was found to be best in inhibiting the fungus. Jadav et al. (2008) reported that Garlic bulb (10 %) extract was effective in inhibiting the growth of *C. gloeosporioides.* Prashanth et al. (2008) evaluated the plant extracts of Eucalyptus, Garlic, Datura, Ocimum and *Polyalthia longifolia* and found that these extracts inhibited the growth of *C.gloeosporoides,* the causal organism of pomegranate anthracnose.

2.8 *In vitro* evaluation of chemicals

The mycelial growth of *Pestalotia palmarum* causing grey blight of coconut was effectively checked *in vitro* by Ziram and Mancozeb (Wilson and Peethambaran, 1971).

Das and Mahanta (1985) tested 14 fungicides for their relative efficacy at 100, 200, 300, 400,500 ppm concentration. They found that Bavistin, Hexathir and Tecto-60 completely inhibited the growth of *P. palmarum* at all concentrations. Rest of the fungicides permitted growth. Growth and sporulation of *P. palmarum* was completely inhibited *in vitro* by Carbendazim (Kudalkar et al., 1991).

Based on the *in vitro* evaluation of fungicides, Anupama (1997) reported that Bordeaux mixture (1000 ppm and 1500 ppm), Carbendazim (2000, 2500 and 3000 ppm) and Mancozeb (2000 and 3000 ppm) gave 100 per cent inhibition of *P. palmarum* causing grey leaf spot disease in coconut where as Captafol at 4500 ppm gave only 75 per cent control and copper oxy chloride at 3500 ppm gave 84.1 per cent control.

Propiconazole was the most effective in inhibiting the mycelial growth of *P. palmarum* at all the concentrations followed by Carbendazim, Hexaconazole, Difenconazole and Tridemorph at 0.1 and 0.15 per cent conc. Among the non-systemic fungicides Mancozeb was most effective in checking the fungal growth at 0.2 and 0.3 per cent followed by copper oxy chloride and least control with Cholrothalonil (Praveena, 1999)

Karthtikeyan and Bhaskaran (1998) revealed that Carbendazim, Thiophanate methyl and Mancozeb inhibited the mycelial growth of *Pestalotiopsis palmarum* completely at the conc. of 500 ppm. Khalequzzaman et al. (1998) reported that Bavistin (Carbendazim), tilt 250 EC, Cupravit and Dithane M-45 (Mancozeb) performed best against *Pestalotia palmarum in vitro*.

Islam et al. (2004) reported that Bavistin (100, 200, 300 ppm) and Tilt (200 and 300 ppm) had completely inhibited the mycelial growth of *Pestalotia*

palmarum, the causal agent of leaf spot of betelnut. He also reported that Dithane M-45 and Ridomil were poor performing fungicides *in vitro* against the same pathogen.

Saju et al. (2011) reported that Carbendazim (0.1 %) and Carbendazim +Mancozeb (0.1 %) inhibited the mycelial growth up to 91.4 % and 79.8 % of *Pestalotiopsis* sp. causing leaf streak in cardamom *in vitro*. Anand et al. (2012) recorded that maximum inhibition of *Pestalotia psidii* causing guava canker was obtained with Benomyl and Carbendazim @ 0.1 %, up to 91.7 % each, *in vitro*.

Das and Mahanta (1985) tested 14 fungicides for their relative efficacy at 100, 200, 300, 400,500 ppm conc. They found that Bavistin, Hexathir and Tecto-60 completely inhibited the growth of *P. palmarum* at all conc.

Benomyl (0.1 %), Captan (0.25 %), Thiram (0.25 %) and Phenyl mercuric urea formulation (0.1 %) were highly fungicidal to mycelial growth of *Colletotrichum gloeosporioides* the causal agent of inflorescence dieback of arecanut (Saraswathy et al., 1975). Systemic RH-2161 and non-systemic Copper oxy chloride, Mancozeb, Zineb, Ziram were effective in inhibiting the growth of the fungus *in vitro* (Hegde and Hegde, 1986).

Mancozeb (0.3 %) and Captafol (0.2 %) were effective when sprayed for four rounds at monthly interval during June to September period to control the leaf spot of arecanut caused by *C. gloeosporioides* and *Phyllosticta arecae* (Ramanujam and Chandramohanan, 1987). Mancozeb, Carbendazim and Bordeaux mixture were similarly effective in controlling leaf blight of arecanut caused by *Phyllosticta arecae* but Mancozeb had the best resistance to withering and persisted longest for 28 days (Bhat et al., 1989).

The complete inhibition of the growth of *C. gloeosporioides* and *Phyllosticta arecae* was recorded in Carbendazim (0.05 %), Mancozeb (0.3 %), Captafol (0.2 %) and Ziram (0.3 %). Systemic fungicide RH-2161 followed by Copper oxy chloride, Zineb, Ziram and Mancozeb were most effective in

inhibiting the mycelial growth of *C . gloeosporioides* causing anthracnose of arecanut (Hegde et al., 1992).

Carbendazim gave the best control of *Colletotrichum catechu*, causing leaf spot of betelnut at all the conc. tested followed by Propiconazole, folicur, fosetyl-Al and Mancozeb at 1000 ppm (Goswami et al., 1996). The arecanut leaf spot causing pathogen *Colletotrichum gloeosporioides* was effectively controlled *in vitro* by Mancozeb (0.2 and 0.25 %), Carbendazim (0.1 and 0.2 %) and Thiophanate methyl (0.05 to 0.15 %) (Padalkar et al.,1996).

Ekbote et al. (1996) reported that among the six fungicides tested, derosal gave cent per cent inhibition of mycelial growth at 0.05 and 0.10 per cent conc., while Indofil M- 45 gave cent per cent inhibition at 0.3 per cent conc. The least per cent inhibition of mycelial growth was observed in kavach at all the tested conc.

Propiconazole at 500 ppm, Carbendazim and Thiophante methyl at 750 ppm, Mancozeb and Chlorothalonil at 1000 ppm and Copper oxy chloride at 2000 ppm were highly effective in inhibiting the growth of *C . gloeosporioides* causing inflorescence dieback of arecanut (Pradeepkumar, 2000).

Prashanth et al. (2008) reported that among the non-systemic fungicides, combi product Carbendazim + Mancozeb recorded highest per cent inhibition of mycelial growth (89.23 %) of fungus, which was followed by Propineb (87.78 %) and the least inhibition of fungus was recorded in Chlorothalonil (53.78 %) at 0.1 per cent conc. Among four systemic fungicides maximum per cent inhibition of growth of *Colletotrichum gloeosporioides* was observed in Difenoconazole (90.78 %) and Propiconazole (90.78 %) which were on par with each other and was followed by Carbendazim (88.89 %) while least per cent inhibition of fungus was recorded in Iprobenfos (75.99 %) at 0.1 per cent conc.

Gud and Raut (2008) reported that, Thiophenate-methyl and Propiconazole were most effective against *Colletotrichum gloeosporioides* followed by Hexaconazole and Carbendazim. Patel (2009) reported that,

Carbendazim, Mancozeb+Carbendazim, Propiconazole, Tricyclazole were on par with each other, which showed 100% effectiveness.

Watve et al. (2009) reported that, Carbendazim (0.1 %), Propiconazole (0.1 %), Difenconazole (0.1 %) and Copper oxychloride (0.3 %) inhibited the growth and sporulation to the extent of 100 % followed by Bordeaux mixture (1 %), Tridemefon (0.1 %), and Mancozeb (0.1 %).

Vinod et al. (2009) reported that Carbendazim was found to be effective among all the tested chemicals and gave cent per cent mycelial inhibition of *Colletotrichum gloeosporioides*

2.9 Compatibility of chemicals and plant extracts with bio control agents

Bheemaraya (2012) recorded that *Trichoderma* spp. is compatible with Neem Seed Kernal Extract (NSKE), Pongamia leaf extract, Eucalyptus leaf extract. Sarkar et al. (2010) reported that Propiconazole completely inhibited the growth of *Trichoderma harzianum* at 300 ppm.

Archana et al. (2012) reported that *Trichoderma viride* is completely compatible with Azoxystrobin 23 SC up to 15 ppm of its conc. They also observed that @ 25 ppm of chemical conc. the biocontrol agent is 42.2% compatible only.

Bheemaraya et al. (2012) observed that Carbendazim, Propiconazole at 0.1 and 0.2 % completely inhibited the growth of *Trichoderma viride* and they are not compatible with the biocontrol agent. Ranganathswamy et al. (2012) observed that Benomyl, Carbendazim, Propiconazole @ 0.1 %, Hexaconazole @ 0.2% and Tricyclazole @ 0.06% were completely inhibitory on *Trichoderma* isolates. But, Azoxystrobin was moderately compatible with least inhibitory effect on radial growth (35.0%) to *Trichoderma* isolates.

2.9 *In vivo* Management

It was reported that spraying of coconut trees with a mixture of copper fungicide and DDT thrice a year cured leaf disease caused by *Pestalotia*

palmarum (Anonymous, 1957). Menon (1959) reported that spraying of Kirthi copper could reduce the seedling blight infection in arecanut nurseries. The spray of 1 % Bordeaux mixture at frequent intervals apart from other cultural practices control nursery diseases of arecanut (Rao and Bavappa, 1961).

Menon (1962) used two sprays of magnesium sulphate, boric acid, tannin and dithane as a proprietary fungicide to control seedling blight caused by *Pestalotia, Phyllosticta, Harmodendron* and *Botryodiplodia.* Lily et al. (1965) recommended Bordeaux mixture spray for Pestalotia blight in coconut. Bordeaux mixture and Fytolan were most effective in the control of coconut leaf blight (Rao et al.,1976).

Joshi and Raut (1992) observed that the severe disease of young clove trees caused by *Pestalotia versicolor* was best controlled by 0.1% Carbendazim (Bavistin) sprays applied three times at 15 days interval. Integrated spraying of Carbendazim, Bordeaux mixture and Chlorothalonil along with other management practices checked Pestalotia leaf spot in coconut (Anxianshu and Hanlianjian, 1994).

Root feeding of Thiophanate methyl or Carbendazim or Tridemorph at 2% reduced the severity of Pestalotia leaf blight of coconut in the field and increased the nut yield (Karthikeyan and Bhaskaran, 1998). Carbendazim (0.1%) was the most effective followed by Mancozeb (0.2 %) in controlling the leaf spot disease of coconut caused by *P.palmarum (*Khalequzzaman et al., 1998). Kalim et al. (2000) reported that Carbendazim acts on the pathogen through inhibition of spindle formation during mitosis.

Khalequzamman et al. (2003) observed that Leaf spot of Sapota caused by *Pestalotia sapotae* L. can be controlled by spraying Bavistin (0.1%), Dithane M-45 (0.2%), Tilt 250 EC (0.05%) and the disease was reduced to 61.78%, 53.31%, 45.80 % respectively over control. Sanjay et al. (2008) reported that grey blight disease of tea caused by *Pestalotiopsis theae* can be effectively controlled in the field by spraying Carbendazim (0.05%) and Mancozeb (0.3%). Two sprays of Bordeaux mixture (1%) once during summer followed by the other in September

effectively controlled the anthracnose disease of arecanut caused by *Colletotrichum gloeosporioides* (Hegde and Hegde, 1986).

Bharadwaj and Thakur (1991) conducted field trials, where they used Carbendazim (0.1 %), Captafol (0.25 %) and Mancozeb (0.25 %) applied alone as single sprays at 60 days, two sprays at 45 and 60 days or three sprays at 45, 60 and 75 days or in sequence one after the other on a three sprays schedule at 45, 60 and 75 days after sowing for the control of leaf spot and pod blight of urd bean, caused by *C. dematium* f. sp. *truncatum*. All three fungicides reduced the disease severity on foliage, however, three sprays schedule was found more effective than the single or two spray Carbendazim than Mancozeb or Captan.

Goswami et al., (1996) revealed that the application of Propiconazole @ 0.2 per cent gave the best control of leaf spot of arecanut caused by *C. arecae* by reducing disease severity of 45.83%. Economic analysis indicated that the net gain over the control was the highest with Mancozeb @ 0.25 per cent followed by fosetyl –Al @ 0.25 per cent.

Desai (1998) conducted field studies in Karnataka and reported that by spray of kitazin at 0.05% to 0.2% gave effective control (97.78-99.5%) of anthracnose caused by *Colletotrichum gloeosporioides* on pomegranate. Jamadar et al. (1998) reported that combi product like Mancozeb 0.2% + Carbendazim 0.05 % was more effective in controlling fruit spot incidence over control followed by Bordeaux mixture, reduced the disease by more than 88% against control.

Navale et al. (1998) evaluated the efficacy of fungicides against the fruit spot of pomegranate in mrigbahar. Ziram at the rate of 0.25 per cent was found to be cheaper than the remaining fungicides tested, which recorded least per cent disease index (0.17%). Charigkapakorn (2000) reported that, crude extract from rhizome, leaves and creeping branches of sweet flag (*Acorus calamus* L.), palmorosa (*Cymbopogon martinii*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil could restrict growth of the anthracnose fungus. Among the biofungicides used against the fungus *Colletotrichum* spp. on chilli fruit,

found that the most effective control was sweet flag crude extract when applied in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage.

Gaikwad (2000) reported that, seven sprays of fungicides Carbendazim (0.1%) and Mancozeb (0.2%) were found to be effective for controlling leaf and fruit spot of pomegranate caused by *Colletotrichum gloeosporioides.*

Chandrasekaran et al. (2000) studied the effect of a plant extract, an antagonist and a fungicide treatment both individually and in combination. Seed treatment with alum (0.1%) recorded the highest seed germination of 90 per cent, compared to 68.0 per cent in the untreated control. Seed treatment followed by a foliar spray with *Lawsonia inermis* leaf extract (1%) and alum (0.1%) recorded leaf anthracnose and pod blight incidence of 7.0 and 4.2 per cent, respectively with a grain yield of 2191 kg ha^{-1}. Seed treatment with *L. inermis* (1%) + *Trichoderma viride* (0.4%) + alum (0.1%) registered 7.4 per cent leaf anthracnose, 5.6 per cent pod blight incidence and yield of 2186 kg ha^{-1}.

Varaprasad (2000) studied on integrated approach for the management of chickpea blight disease. Out of six treatments tested, seed treatment with Carbendazim @ 2 g per kg + two foliar spray of SAAF (0.05%) at 15 days interval gave maximum reduction in disease incidence followed by seed treatment with Carbendazim @ 2 g per kg + foliar spray with SAAF 0.05% and *Polyalthia longifolia* (10%) extract at 15 days interval.

Deeksha and Tripathi (2002) studied on management of blackgram anthracnose and found that seed treatment followed by two prophylactic sprays of Bavistin or Tilt @ 0.1% each at 15 days interval showed minimum disease severity and maximum grain yield followed by Contaf (0.1%) and Indofil M-45 (0.2%) sprayed plots. Among the biocontrol agents, *Gliocladium virens* gave better results than *Trichoderma harzianum.*

Chhata and Kumawat (2001) studied the management of bacterial and fungal fruit spot of pomegranate two years on farmer field and reported that four

sprays of Bavistin (0.1%) + Streptocycline (0.04%) at an interval of 15 days reduced the PDI to 10.42 whereas untreated control recorded 71.21 PDI. Madhusudhan (2002) observed that either Benomyl or Carbendazim seed treatment at the rate of two grams per kg of seed along with two foliar applications at 0.1 per cent on 30 and 45th day of sowing was found effective in controlling the soybean anthracnose.

Strobilurin fungicides Azoxystrobin (Quadris), Trifloxystrobin (Flint), and Pyraclostrobin (Cabrio) have recently been labeled for the control of anthracnose of chilli (Alexander and Waldenmaier, 2002; Lewis and Miller, 2003). Laxman (2006) studied the efficacy of different fungicides and biorationals under field condition against green gram anthracnose and found that among different treatment, the least disease incidence was observed in Propiconazole followed by Hexaconazole and Carbendazim. But, in case of biorationals, the least disease incidence was noticed in azadirachtin.

Prasanna Kumar et al. (2006) reported that, Carbendazim (0.1%) treatment showed lowest per cent disease index followed by Tricyclazole, Benomyl and Copper oxychloride against *Colletotrichum gloeosporioides* of mango anthracnose. Prashanth et al. (2008) reported the efficacy of Difenoconazole, Propiconazole and Iprobenfos against *Colletotrichum gloeosporioides* in managing the anthracnose of pomegranate. Navale et al. (2009) reported that, spraying of 0.1% Difenoconazole against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate showed least per cent disease intensity, maximum per cent disease control and highest fruit yield followed by 0.1 % Propiconazole. Patel et al. (2009) reported that, Carbendazim sprayed fruits showed highest per cent disease control over unsprayed fruits and Propineb showed lowest per cent disease control against *Colletotrichum gloeosporioides* of pomegranate. Ajit Kumar Singh (2009) reported that spray of SAAF (Mancozeb + Carbendazim) @ 0.25% and Carbendazim @ 0.1% effectively controlled the leaf spot of turmeric caused by *Colletotrichum capsici.*

Materials and methods

3. MATERIALS AND METHODS

The present investigation entitled ''Management of leaf spot diseases of Arecanut (*Areca catechu* L.)" was carried out in the Department of Plant Pathology, College of Agriculture, Vellayani during 2011-2013.

3.1 GENERAL PROCEDURES

3.1.1 Glassware and cleaning

For all the laboratory studies, Corning and Borosil made glasswares were used. The glasswares were kept in boiling water for 30 min. Then, they were scrubbed thoroughly with a detergent followed by cleaning in tap water and finally rinsed in distilled water and dried.

3.1.2 Sterilization

All the glasswares and the media used in the studies were sterilized in the autoclave at 121°C / 15 Psi for 15 min.

3.1.3 Sterilization of laminar air flow chamber

The isolation and sub culturing works were conducted under aseptic conditions in laminar air flow cabinet. The laminar flow chambers was switched on and after 10 min, the hands and working place were sterilized by swabbing with 70 % alcohol (ethanol). In case of sterilized glasswares, the mouth of the flasks, test tubes and petriplates were opened and closed near the flame during various culture and subculture procedures.

3.1.4 Culture medium

For the experimental studies standard culture medium like Potato Dextrose Agar (PDA), Nutrient Agar (NA), Rose Bengal Agar (RBA) and Plain Agar one per cent were used.

3.2 Isolation and identification of the fungus

The Arecanut leaves showing leaf spot or blight symptoms were collected from Arecanut gardens located in Instructional Farm at Vellayani, Peringamala in Thiruvananthapuram and Balussery in Calicut district. These specimens were preserved in the refrigerator at 5±1° C and also by dry preservation.

3.2.1 Collection of infected specimens

The fungus was isolated from the infected leaves of arecanut following the standard procedures. The infected diseased samples along with healthy tissues were cut into small pieces measuring 0.5 mm to one mm and surface sterilized by dipping in 0.1 % Mercuric chloride ($Hgcl_2$) solution for one min. The treated plant tissues were washed three times with sterilized distilled water. Excess water was decanted by soaking with sterilized blotting paper.

The cut pieces were then placed into sterilized PDA in petri dishes (three pieces / dish). These plates were incubated at a temperature of 27 ±1°C and the growth was examined four to five days after incubation. The fungus from such plates was purified using single spore isolation method. (Rangaswami and Mahadevan,1999)

3.2.2 Single spore isolation

Ten ml of clear filtered two per cent water agar solution was poured into sterile petri plates and allowed to solidify. The dilute spore suspensions were prepared in sterile distilled water from ten days old culture. Two ml of spore suspension was spread uniformly on water agar plates. After four hours of incubation at 27±1°C, the plates were examined to locate the germinated conidia. Single isolated germinated conidium was marked with ink on the glass surface of the agar and transferred to PDA slants in such a way that the conidium bearing surface was in contact with PDA surface and incubated at 27±1°C. The pure

culture was sub cultured once in a month and preserved in refrigerator and used for further studies.

3.2.3 Maintenance of the cultures

The fungi obtained were sub cultured on PDA slants and allowed to grow at 27±1°C for 12 days. Such slants were preserved in refrigerator at 5°C and maintained. Sub culturing was done once in a month and such cultures were used throughout the study. Virulence of the fungi was maintained by inoculating the isolates onto the host and re-isolation after every three months.

3.2.4 Identification of the fungi

The morphological features of fungus such as mycelial and cultural characters, length and breadth of conidia, fruiting body were studied by using the microscope.

3.2.5 Proving the pathogenicity

The healthy young leaves were selected from the areca seedlings, washed thoroughly with tap water, swabbed with 0.1% mercuric chloride and washed with sterile distilled water. Injuries were made on the leaflets using sterile needles. Fungal mycelium from seven days old culture was harvested and placed on the wound, cotton moistened with sterile water was placed above the mycelial mat. The leaves placed with moistened cotton without fungal cultures above the wound served as the control. Symptom development was observed closely and keenly. The organism was re-isolated from these artificially inoculated leaves and the culture so obtained was compared with original culture as per Koch's postulates (Koch Robert, 1893).

3.3 Characterization of the pathogens

3.3.1 Morphological and cultural characters of the pathogens

Identification of the pathogens was done by studying their growth in the culture medium and spore morphology using microscope. For identification and

deposition, cultures were sent to ARI (Agharkar Research Institute), NFCCI (National Fungal Culture Collection of India), Pune.

3.3.2 Molecular characterization of leaf spot pathogens.

In the present study an attempt was made to analyse the ITS sequences of *Pestalotiopsis palmarum*, *Colletotrichum gloeosporioides* and *Phomopsis palmicola* by considering various parameters viz. sequence similarity by using multiple alignment and phylogenetic tree to get the correct conclusion regarding the identification of the *P. palmarum*, *C. gloeosporioides* and *P. palmicola*.

The cultures were submitted to RGCB, Thiruvananthapuram (Rajiv Gandhi Centre for Biotechnology) for sequencing. The protocol they followed was as follows.

3.3.2.1 Isolation of DNA has been done using Gen Elute Plant Genomic DNA Mini prep Kit (Sigma)

The tissue/mycelium (about 50 mg) was transferred to a micro centrifuge tube and ground in 350 µl of lysis solution A and 50 µl of lysis solution B using a micro pestle. The mixture was incubated at 65°C for 10 min with occasional inversion. 130 µl of precipitation solution was added to the mixture, mixed completely by inversion and the sample was placed on ice for five min. The sample was centrifuged at 14,000 rpm (Eppendorf Centrifuge 5804 R) for five min to pellet the cellular debris, proteins, and polysaccharides. The supernatant was transferred to the Gen Elute filtration column tube and centrifuged at 14,000 rpm for one min. This removed any cellular debris not removed in the previous step. The filtration column was discarded and 700 µl of binding solution was added directly to the flow through liquid and mixed thoroughly by inversion. 700 µl of this mixture was added into Gen Elute nucleic acid binding column and centrifuged at 14,000 rpm for one min.

The flow through liquid was discarded and the collection tube was retained. The column was returned to the collection tube and the remaining sample was applied to the column. Centrifugation was repeated as above and the flow through liquid and the collection tube were discarded. The binding column was placed into a fresh two ml collection tube. 500 µl ethanol-added wash solution was added to the binding column and centrifuged at 14,000 rpm for one min. The flow through liquid was discarded and the collection tube was retained. The wash was repeated once more. The binding column was transferred to a new collection tube. 30 µl of elution solution (pre-warmed to 65°C) was added to the binding column and centrifuged at 14,000 rpm for one min. The stock DNA was properly labelled and stored at 4 °C.

3.3.2.2 Agarose Gel Electrophoresis for DNA Quality check

The quality of the DNA isolated was checked using agarose gel electrophoresis. One µl of 6X gel-loading buffer (0.25 % bromophenol blue, 30 % sucrose in TE buffer pH-8.0) was added to five µl of DNA. The samples were loaded to 0.8 % agarose gel prepared in 0.5 X TBE (Tris-Borate-EDTA) buffer containing 0.5 µg/ml ethidium bromide. Electrophoresis was performed with 0.5 X TBE as electrophoresis buffer at 75 V until bromophenol dye front has migrated to the bottom of the gel. The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.2.3 PCR Analysis

PCR amplification reactions were carried out in a 20 µl reaction volume which contained 1X PCR buffer (100 mM Tris HCl, pH-8.3; 500 mM KCl), 0.2 mM each dNTPs (dATP, dGTP, dCTP and dTTP), 2.5 mM $MgCl_2$, 20ng DNA, one unit of AmpliTaq Gold DNA polymerase enzyme, 0.1 mg/ml BSA and 4% DMSO, 5 pM of forward and reverse primers.

Primers used

Target	Primer Name	Direction	Sequence (5' → 3')	Reference/Remarks
ITS	ITS-1F	Forward	TCCGTAGGTGAACCTTGCGG	White et al., 1990
	ITS-4R	Reverse	TCCTCCGCTTATTGATATGC	

The PCR amplification was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems).

PCR amplification profile

ITS

95 °C - 5.00 min

95 °C - 0.30 min
58 °C - 0.40 min
72 °C - 1.00 min
} 40 cycles

72 °C - 5.00 min
4 °C - ∞

3.3.2.4 Agarose Gel electrophoresis of PCR products

The PCR products were checked in 1.2 % agarose gel prepared in 0.5 X TBE buffer containing 0.5 µg/ml ethidium bromide. 1 µl of 6 X loading dye was mixed with five µl of PCR products and was loaded and electrophoresis was performed at 75 V power supply with 0.5 X TBE as electrophoresis buffer for about 1-2 hours, until the bromophenol blue front had migrated to almost the

bottom of the gel. The molecular standard used was 2-log DNA ladder (NEB). The gels were visualized in a UV transilluminator (Genei) and the image was captured under UV light using Gel documentation system (Bio-Rad).

3.3.2.5 ExoSAP-IT Treatment

ExoSAP-IT (GE Healthcare) consists of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), in a specially formulated buffer for the removal of unwanted primers and dNTPs from a PCR product mixture with no interference in downstream applications.

Five micro litres of PCR product was mixed with two µl of ExoSAP-IT and incubated at 37°C for 15 min followed by enzyme inactivation at 80°C for 15 min.

3.3.2.6 Sequencing using BigDye Terminator v3.1

Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufactures protocol.

The PCR mix consisted of the following components:

PCR Product (ExoSAP treated)	-	10-20 ng
Primer	-	3.2 pM (either Forward or Reverse)
Sequencing Mix	-	0.28 µl
5x Reaction buffer	-	1.86 µl
Sterile distilled water	-	make up to 10 µl

The sequencing PCR temperature profile consisted of a 1st cycle at 96°C for two min followed by 30 cycles at 96°C for 30 sec, 50°C for 40 sec and 60°C for four min for all the primers.

3.3.2.7 Post Sequencing PCR Clean up

1. Make master mix I of 10 µl milli Q and 2 µl 125 mM EDTA per reaction
2. Add 12 µl of master mix I to each reaction containing 10 µl of reaction contents and are properly mixed.
3. Make master mix II of 2 µl of 3 M sodium acetate pH 4.6 and 50 µl of ethanol per reaction.
4. Add 52 µl of master mix II to each reaction.
5. Contents are mixed by inverting.
6. Incubate at room temperature for 30 min
7. Spin at 14,000 rpm for 30 min
8. Decant the supernatant and add 100 µl of 70 % ethanol
9. Spin at 14,000 rpm for 20 min.
10. Decant the supernatant and repeat 70 % ethanol wash
11. Decant the supernatant and air dry the pellet.

The cleaned up air dried product was sequenced in ABI 3500 DNA Analyzer (Applied Biosystems).

3.3.2.8 Sequence Analysis

The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010).

3.3.2.9 Bioinformatics

The obtained ITS sequences of all the leaf spot pathogens, were searched in the NCBI (National centre for Biotechnology Information) database using BLAST (N) (Basic Local Alignment Search Tool (Nucleotide)) and based on

genetic similarity the organisms has been identified. The sequences were submitted to the NCBI's GenBank for accession numbers. The sequences were put into Clustal omega database for obtaining the phylogenetic tree using neighbour joining method and Multiple alignment of ITS region of *Pestalotiopsis palmarum*, *Colletotrichum gloeosporioides* and *Phomopsis palmicola.*

3.3.3 Growth of fungal cultures on different solid media

The cultural characters of the fungi were studied on the following ten different solid media and the best media for growth was identified.

1. Potato dextrose agar
2. Oat meal agar
3. Host extract agar
4. Czapek's agar
5. Malt extract agar
6. Sabouraud's agar
7. Yeast extract agar
8. Richards' agar
9. Potato carrot agar
10. Corn meal agar

The composition and preparation of the above mentioned synthetic and non-synthetic media were obtained from Ainsworth and Bisby's Dictionary of the fungi (Hawksworth et al., 1983). The composition of the media is given in Appendix1.

Twenty ml of each medium listed above was poured aseptically into 90 mm dia Petri plates. After solidification, five mm discs of fungi from actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of Petri dish. Each set of experiment was replicated thrice and the plates

were incubated at 27±1°C. The measurements of the colony diameter were taken when the maximum growth was attained in any one of the media tested. Then, cultural characters such as colony diameter, colony colour and type of margin were also recorded.

3.3.4 Effect of pH on growth of the fungal isolates

The fungi were grown in PDA medium with selected pH range of 5.0, 6.0, 6.5, 7.0, and 8.0. The pH levels were adjusted by adding 1 N alkali (NaOH) or acid (HCl). Seven day old five mm mycelial discs from actively growing culture were inoculated in the centre of the petri dishes. They were incubated at 27±1°C. Observations on radial growth of the fungi were taken when the fungi attained full growth in any one of the pH levels tested.

3.4. Survey on the incidence and intensity of the disease.

3.4.1 Assessment of the intensity of leaf spot disease of arecanut

A survey was conducted to study the incidence of the leaf spot disease in the Instructional farm, Vellayani. Randomly eighty plants were selected and scoring was done to access the disease intensity.

3.4.1.1 Scoring of disease intensity and calculation of PDI

The scoring of leaf spot disease was done based on 0 to 5 severity scale (Bhat, 1983) as described in Table 1. Disease assessment was made for each leaf starting from apical spindle leaf to the bottom leaf. In each leaf, disease assessment was made on one leaflet each at the base, middle and apical portion of leaf.

Based on this scale, the per cent disease index was calculated using the formula (Wheeler, 1969).

$$PDI = \frac{\text{Sum of all individual scores}}{\text{Total no. of observations} \times \text{Maximum score}} \times 100$$

Table 1: Disease score chart for leaf spots of Arecanut

Leaf area affected by leaf spots	Disease score
0 per cent of the leaflet covered by leaf spot	0
1-5 per cent of the leaflet covered by leaf spot	1
6-15 per cent of the leaflet covered by leaf spot	2
16-30 per cent of the leaflet covered by leaf spot	3
31-50 per cent of the leaflet covered by leaf spot	4
> 50 per cent of the leaflet covered by leaf spot	5

3.4.2 Assessment of the incidence of leaf spot disease of arecanut

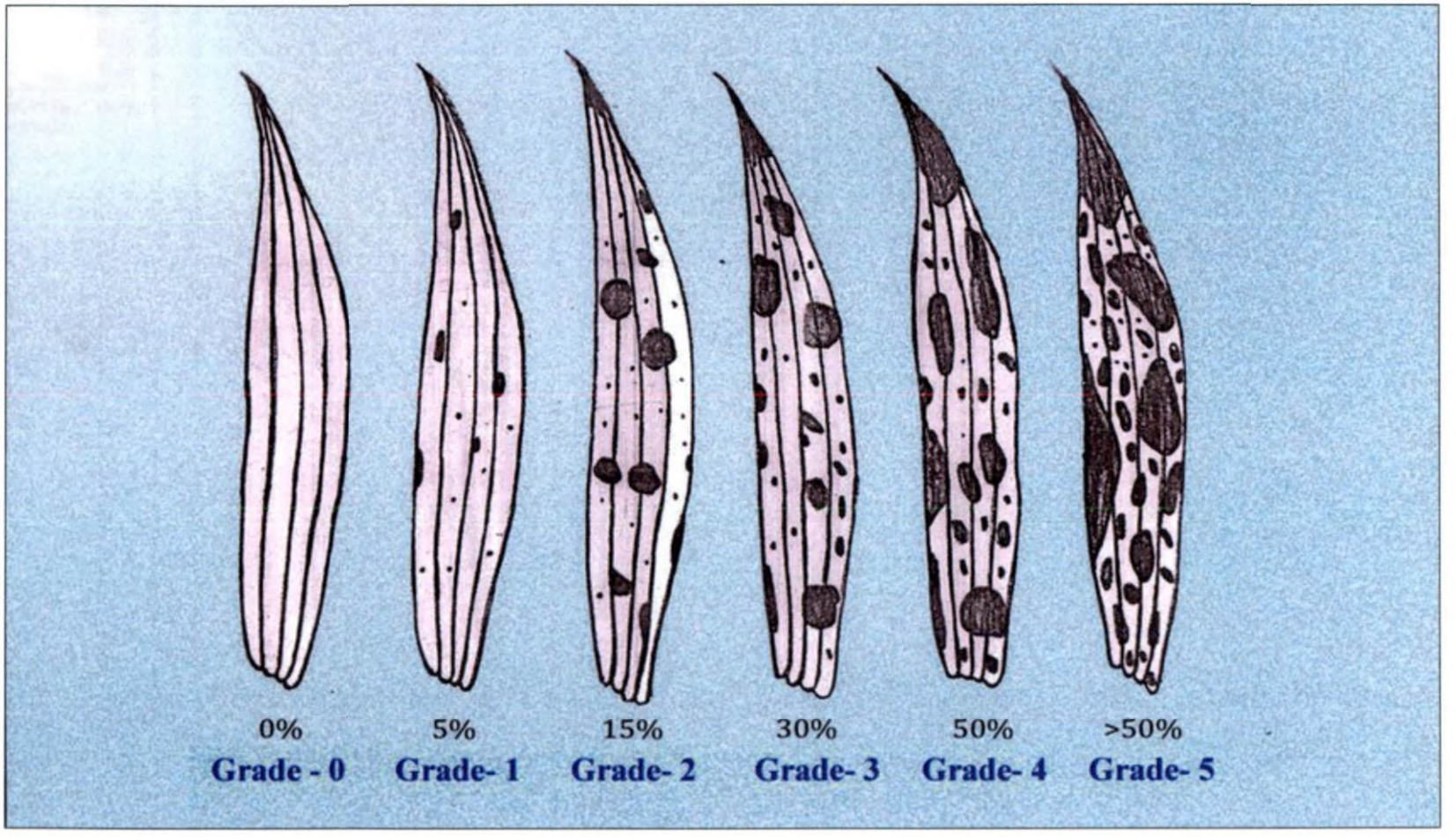
Survey on incidence of leaf spot was observed in Areca plantations in Instructional Farm at Vellayani, Peringamala in Thiruvananthapuram and Balussery in Calicut district. Disease incidence was assessed by counting the number of infected palms and the total number of palms observed which are below 10 years age. Incidence of the disease (%) was calculated using the formula.

$$\text{Disease Incidence (\%)} = \frac{\text{No. of infected palms}}{\text{Total no. of palms observed}} \times 100$$

3.5 Effect of weather factors on the leaf spot disease of arecanut

To study the influence of weather factors on the development and spread of disease, six plants were selected and marked in the Instructional Farm, Vellayani. Disease was scored for one year from April 2012 to March 2013 at monthly intervals. Scoring was done at the end of the month so the cumulative effect of weather factors on the disease for that month can be well studied.

Fig 1: **Disease score chart for leaf spots of arecanut palms**



The weather parameters like maximum temperature, minimum temperature, relative humidity and rainfall data were collected from the Department of Meteorology, College of Agriculture, Vellayani.

3.6 Isolation of antagonistic organisms from phyllosphere and rhizosphere of healthy areca palms

The leaf and soil samples were collected from the healthy areca palms growing in the Instructional Farm, Vellayani. Isolation was done by following serial dilution technique (Aneja, 2003).

3.6.1 Isolation of antagonists from the phyllosphere

Ten gram of leaf sample was weighed and cut into small bits and added to a conical flask containing 99 ml of water, for 10^{-2} dilution and shaken for 10 min. From this 0.1 ml aliquot was transferred into a nine ml test tube and subsequently from this tube 0.1 ml was transferred into a another test tube containing nine ml of aliquot. So the dilutions in the test tube were 10^{-3} and 10^{-4} dilutions. Subsequently 10^{-5}, 10^{-6} dilutions were prepared. From 10^{-3} and 10^{-4}, 0.1 ml aliquot was transferred into petriplates containing Martin's Rose Bengal Agar medium and *Trichoderma Specific Medium* (TSM) by following spread plate technique.

From 10^{-5} and 10^{-6} , 0.1 ml was transferred into plates containing Nutrient Agar medium and incubated at room temperature.

3.6.2 Isolation of antagonists from the rhizosphere

Ten gram of rhizosphere soil sample was weighed and added into a conical flask containing 99 ml of water, for 10^{-1} dilution and shaken for 10 min. From this solution it was serially diluted up to 10^{-6} dilution. From 10^{-3} and 10^{-4} dilutions 0.1 ml was added into Martin's Rose Bengal Agar medium and Trichoderma Specific Medium and also a pinch of soil was added into TSM. For bacteria 0.1 ml aliquot from 10^{-5} and 10^{-6} was added into NA medium and incubated.

3.7 *In vitro* evaluation of biocontrol agents, botanicals, chemicals and compatibility studies.

3.7.1 *In vitro* evaluation of biocontrol agents

The efficacy of bioagents was tested against identified fungal pathogens *P. palmarum* and *C. gloeosporioides* for radial growth inhibition in the PDA medium using dual culture technique.

Dual culture test

The fungal bioagents and the test fungus were inoculated opposite to each other and placed one cm away from the periphery in a single petri plate.

The bacterial bioagents were streaked four cm apart inside the plate along side of the test fungus which was inoculated in the centre of the plate.

Five replications were maintained for each treatment, control was maintained by inoculating pathogen alone in petri plates. They were incubated for seven days. The dia of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by the formula of Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of growth of the test fungus

C= Radial growth of the pathogen in control

T = Radial growth of the pathogen in treatment (Botanicals / Fungicides / Bioagents)

3.7.2 *In vitro* evaluation of botanicals against pathogens

The present investigation was carried out to evaluate the extracts of five plant species to know the presence of fungitoxicant properties against *Colletotrichum gloeosporioides* and *Pestalotiopsis palmarum*

Preparation of plant based products

100 g of fresh healthy plant parts (leaves/bulb) were collected and washed with distilled water, then ground in 100 ml of sterile water. The product was filtered through muslin cloth and collected filtrate was centrifuged @ 6000 rpm for 25 min at 25°C. The supernatant obtained was poured into the funnel through Whatman No.42 filter paper. The obtained solution was fed into membrane filter unit for sterilization. The filtrate was collected in aseptic condition in the sterilized screw capped bottle. This solution was considered as 100 % concentrate.

The botanicals were tested against the fungi following poisoned food technique (Shravelle, 1961). Different concentrations (see Table 2) of the botanicals were prepared and added to the double strength PDA. These were poured 15 ml each in sterilized petri dishes. Five mm size discs were cut from the five days old actively growing culture of the pathogen and inoculated in the centre of the petriplates, control plates were maintained by adding sterile distilled water in double strength PDA. The plates were incubated at 27±1°C for seven days. Three replications were maintained for each treatment. The colony dia was measured in two directions and average was recorded. Per cent inhibition of mycelial growth of the fungus was calculated using the formula of Vincent (1947).

Table 2: Botanicals and their concentrations used for *in vitro* evaluation.

SI. No.	Plants (Common name)	Scientific name	Plant part used	Concentrations (%)
1	Datura	*Datura stramonium*	Leaf	10, 20, 30
2	Mari gold	*Tagetes erecta*	Leaf	10, 20, 30
4	Bougainvillea	*Bougainvillea spectabilis*	Leaf	10, 20, 30
3	Neem	*Azadirachta indica*	Leaf	2, 4, 6
5	Garlic	*Allium sativum*	Bulb	2, 4, 6

3.7.3 *In vitro* evaluation of fungicides

The efficacy of six fungicides was tested against *C. gloeosporioides* and *P. palmarum* for growth inhibition on the PDA medium following Poisoned food technique (Shravelle, 1961) under *in vitro* condition. The per cent inhibition of growth of the test fungus was calculated by the formula of Vincent (1947). All the fungicides were tried at their recommended dosages, half of the recommended dosage and one fourth of the recommended dosage. The list of fungicides used along with their chemical, trade names and recommended dosages are given in Table 3.

3.7.4 Compatability of chemicals and botanical with biocontrol agent

The effective chemicals and botanicals obtained were tested with biocontrol agent for their compatibility following Poisoned food technique (Shravelle, 1961). Fifty ml double strength PDA medium was prepared and sterilized 50 ml of water was added to another flask and sterilised. Required quantity of chemical / botanical was added into the flask containing water. This was mixed with flask containing double strength PDA medium. The medium was poured into the petriplates and biocontrol agent was inoculated in the centre of the plate. One control was

maintained where only water was added in the medium. Observations were taken when the biocontrol agent attained full growth in the control plate. Per cent inhibition of biocontrol agent by chemical / botanical was calculated using the formula of Vincent (1947).

Table 3: Fungicides and their concentrations used for *in vitro* evaluation

Sl. No	Fungicide common name	Chemical name	Trade name	Recommended Dosage(R.D) (%)	Half of R.D (%)	One fourth of R.D(%)
1	Copper hydroxide	Copper hydroxide	Hi-Dice 77 WP	0.25	0.125	0.062
2	Difenoconazole	Trans,cis-3-chloro-6-[6-methyl-2-(1H-1,2,6-tiazole-1-groupmethyl)-1,3-dioxapentane-2group]phenyl-6 chlorophenyl ether	Score 25 EC	0.1	0.05	0.025
3	Hexaconazole	(RS-2-(2,6-dicholrophenyl-4prophyl,3-dioxolany 2-yl)methyl)-1H-1,2,6-triazole	Contaf plus 5 EC	0.1	0.05	0.025
4	Azoxystrobin	Azoxystrobin	Amistar 23 EC	0.1	0.05	0.025
5	Propiconazole	1-{2-(2,6-dichlorophenyl)-6-1 propyl-1 -1, 3- dioxolan-2-yl}-16-1,2,6-triazole	Tilt 25 EC	0.075	0.037	0.019
6	Mancozeb	Manganese ethylene bisthiocarbamate plus zinc	Indofil M-45	0.3	0.15	0.075
7	Carbendazim	Methyl 2 Benzimidazole Carbamate	Bavistin 50 WP	0.2	0.1	0.05

3.8 *In vivo* management trials

The following were the treatments (Table 4) for the *in vivo* management of leaf spot diseases of arecanut

Table 4: Treatments for management of leaf spot diseases on Arecanut seedlings and young palms

SI.No	Treatments	Concentration (%)
1	T1 - Propiconazole	0.075
2	T2- Carbendazim	0.1
3	T3- Difenoconazole	0.1
4	T4- Azoxystrobin	0.1
5	T5- Garlic (*Allium sativum* L)	6
6	T6- *T. harzianum*	2
7	T7-Azoxystrobin + *T. harzianum*	0.1 + 2
8	T8- Garlic + *T. harzianum*	6 + 2
9	T9- control	No spray

3.8.1 Management trial on seedlings

Design: Completely Randomized Design (CRD)

Treatments: 9

Replications: 7

Data analysis: ANCOVA (Analysis of covariance) with pre-spray data as the covariate.

Transformation applied: Square root transformation

Areca seedlings below one year age were selected and artificially inoculated with the pathogens *C. gloeosporioides* and *P. palmarum.* Seedlings were kept in green house condition during the study. The best biocontrol agent, botanicals and chemicals and their combination obtained in *in vitro* trials were evaluated against the disease to find their effectiveness.

For artificial inoculation of the seedlings, the pathogens *C. gloeosporioides* and *P. palmarum* were multiplied on PDA. The spores were harvested from ten days old culture by scraping the fungal growth with sterilized surgical blade and transfer the same to 50 ml sterilized water contained in a conical flask under aseptic conditions. The flasks were agitated on a rotary shaker for 15 min, so as to get uniform spore suspension and also to break the mycelial clumps if any. The spore suspensions of *C. gloeosporioides* and *P.palmarum* were inoculated at $1x10^5$ spores / ml

Young and old healthy leaves of seedlings were inoculated. Before inoculation these leaves were washed with sterile distilled water and later with 70 per cent ethanol in order to sterilise the surface of the leaf. Then these leaves were pricked with sterilized sharp pins and were inoculated by rubbing with cotton swabs dipped in spore suspension of *C. gloeosporioides* and *P.palmarum.* The leaves rubbed with only sterile distilled water first and later with 70 per cent ethanol was maintained as control plants. Observations were recorded on the time taken for symptom development and the size of the leaf spots developed.

First spray was given at the initiation of disease development. Disease development was scored before spray, 15 days after 1^{st} spray, 15 days after 2^{nd} spray and 30 days after 2^{rd} spray. PDI was calculated and the data were analysed.

3.8.2 Field experiment in the existing young areca palms

Design: Randomized Block Design (RBD)

Treatments: 9

Replications: 6

Data analysis: ANCOVA (Analysis of covariance) with pre-spray data as the covariate.

Transformation applied: Square root transformation

The same treatments were further evaluated on young areca palms (6-8yrs old) grown in the I.F. Vellayani. The palms were selected which were already containing leaf spots on it. Spraying were given at 15 days interval for three times and the disease was scored before spray, 15 days after 1^{st}, 2^{nd} , 3^{rd} spray and 30 days after 3^{rd} spray on the young areca palms. PDI was calculated and data were statistically analysed.

Results

4. RESULTS

The results of the experiments conducted on various aspects of leaf spot diseases of Arecanut (*Areca catechu* L.) during the period 2012 to 2013 with survey and surveillance of diseases; cultural, morphological, physiological aspects of the pathogens; molecular characterization of the pathogens; management of disease by fungicides, bioagents, botanicals and results so obtained are presented hereunder.

4.1 Isolation, purification and proving pathogenicity of the pathogens

4.1.1 Isolation of the pathogen

Standard tissue isolation technique was followed to obtain pathogens from the arecanut leaf spots. Samples were collected from areca palms grown in I.F. Vellayani, Peringamala (TVM) and plantations in Balussery (Calicut).

4.1.2 Purification of the fungi

Three fungal isolates viz., (1, 2 & 3) were purified by single spore isolation

4.1.4 Proving pathogenicity

Artificial inoculations of three fungal isolates obtained viz., isolates (1, 2 & 3) were done separately on healthy arecanut seedlings.

The inoculated leaves with isolate '1' showed typical minute brown spots four days after the inoculation on the wounded leaves, no symptoms were observed on the unwounded leaves. After eight days the spots were enlarged with ashy brown centre surrounded by brown margin (Plate 1).

The fungal isolate '2' produced brown lesions after four days of inoculation with the mycelial mat on the wounded leaves. On the ninth day the spots have enlarged as brown spots with yellow halo surrounding it (Plate 2).

The isolate '3' produced the typical minute water soaked lesions which appeared four days after inoculation with spore suspension on the wounded leaves. Typical brown coloured leaf spots were observed seven days after inoculation. Gradually these spots enlarged elliptically with light grey or straw coloured centre. Pycnidia were also noticed (Plate 3).

Plate 1: Symptoms produced by *Pestalotiopsis palmarum on* artificial inoculation

Plate 2: Symptoms produced by *Colletotrichum gloeosporioides on* artificial inoculation

Plate 3: Symptoms produced by *Phomopsis palmicola on* artificial inoculation

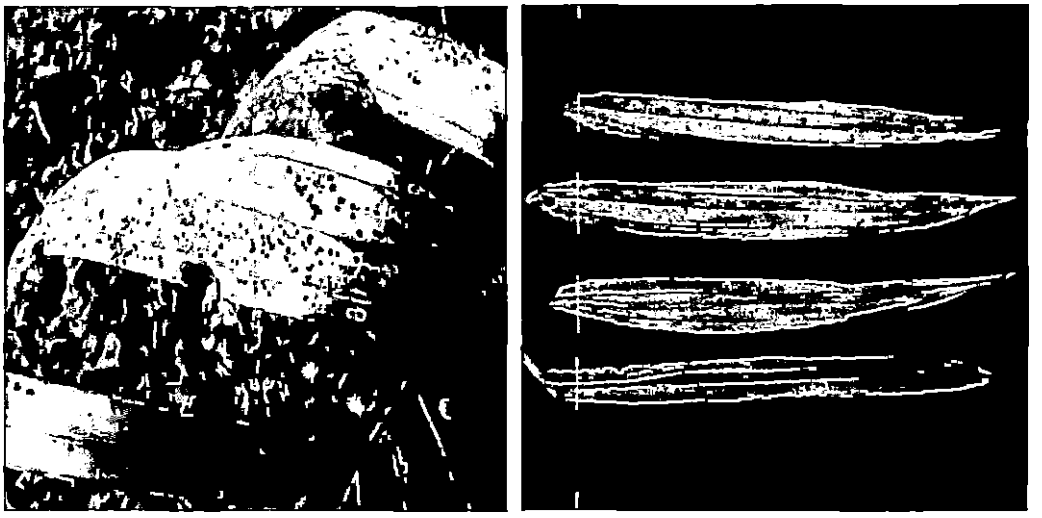


Plate 4: Symptoms produced by *Pestalotiopsis palmarum*

The symptoms produced by all the pathogens were the same to the symptoms observed on the host.The fungi were reisolated from the spots and compared with the original culture proving the pathogenicity of the cultures. Then these cultures were maintained on PDA. Based on characters and spore morphology of the fungus, the isolates '1', '2', '3' were identified as *Pestalotiopsis palmarum*, *Colletotrichum gloeosporioides* and *Phomopsis palmicola*. . These cultures were send to ARI (NFCCI), Pune and they were identified as *Pestalotiopsis sp* and *Colletotrichum gloeosporioides*. and deposited in NFCCI Pune (Accession numbers - 3152, 3154 for *C. gloeosporioides* and 3153 for *Pestalotiopsis* sp.).

4.2 Symptomatology of the diseases

4.2.1 Leaf spot by *Pestalotiopsis palmarum*

Symptoms were noticed initially as spots of 0.3 to 1.0 cm in dia , round to oval and brown to dark brown. The margin of the spot was characterized by dark brown band. Later these were enlarged to 1.2 to 2.5 cm in dia and coalesced to give blighted appearance. The centre of the spot turned to straw or grey colour. On the adaxial surface numerous slightly raised fruiting bodies (acervuli) were found (Plate 4).

4.2.2 Leaf spot by *Colletotrichum gloeosporioides*

The initial symptoms of the disease appeared as small, circular or oblong to irregular brownish spots. The centre of the spots turned light grey or straw colour without any outer ring, some plants were showing brown or grey spots surrounded by an yellow halo. In the advanced stages spots coalesced to give a blighted appearance from tip downwards (Plate 5).

Symptoms of both *P. palmarum* and *C. gloeosporioides were* restricted to the leaves of outer whorls in the palms below 10 years old and older leaves were more prone to the disease.

4.2.3 Leaf spot by *Phomopsis palmicola*

The symptoms of *Phomopsis palmicola* leaf spot on arecanut leaf were more or less similar to that of *Colletotrichum* leaf spot (Plate 6). Initially the spots were more or less round to oval with brownish margin and area of 3-5 mm in size. Usually the leaf spots were confined to the leaf tip and on coalescence extended inwards up to 15 cm from the tip. Centre of the leaf spot was light brown or straw coloured with abundant pycnidia on both surfaces of the leaf but with higher density on upper surface. The border of the spot was

Plate 5: Symptoms produced by *Colletotrichum gloeosporioide*

Plate 6: Symptoms produced by *Phomopsis palmicola*

characterized with a dark brown band of 5 mm width. In advanced stages withering and shredding off of the infected leaf tissue was noticed leading to pre-mature death of whole leaflet.

4.3 Characterization of the pathogens

4.3.1 Morphological and cultural characters of the pathogens

4.3.1.1 *Pestalotiopsis palma*rum

On PDA the isolates produced greyish to white cottony zonate mycelium and black coloured fruiting bodies (acervuli) on the mycelial mats after 4-5 days of inoculation. Colony achieved full growth on the Petri plate in seven days (Plate 7).

The spores were typically five celled and intermediate cells were coloured with constrictions at dividing septa. Conidia measured 20.00 to 25.00 µm in length × 6.0 to 7.5 µm in width with an average of 23.5 µm × 6.5 µm. The upper end of the conidial cell bears two to three long slender, colourless and simple appendages. Their length varied from 15 to 25 µm with an average of 20 µm (Plate 8).

4.3.1.2 *Colletotrichum gloeosporioides*

On PDA the pathogen produced dense, cottony, dirty white to greyish mycelium with even margin. It produced abundant aerial mycelium in the centre of the colony without any zonation. Later it produced conidiophores arising singly or closely packed together in rows (Plate 9).

Conidiophores were single celled, hyaline and aseptate with one or several conidial scars. It produced acervuli on the culture as red - pinkish droplets. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends and with one or two oil globules. Conidia measured 11.48 - 20.01 µm in length × 4.25- 6.62 µm in length and the average being 15.74 × 5.43 µm (Plate 10).

Acervuli produced on the host were disc or cushion shaped, sub-epidermal with light brown coloured setae or spines, which are scattered among conidiophores. Conidiophores were simple, filiform measuring 10 to 12.5 µm. Conidia measured 12.78 µm in length × 4.52 µm in width, one celled, hyaline and cylindrical with rounded ends.

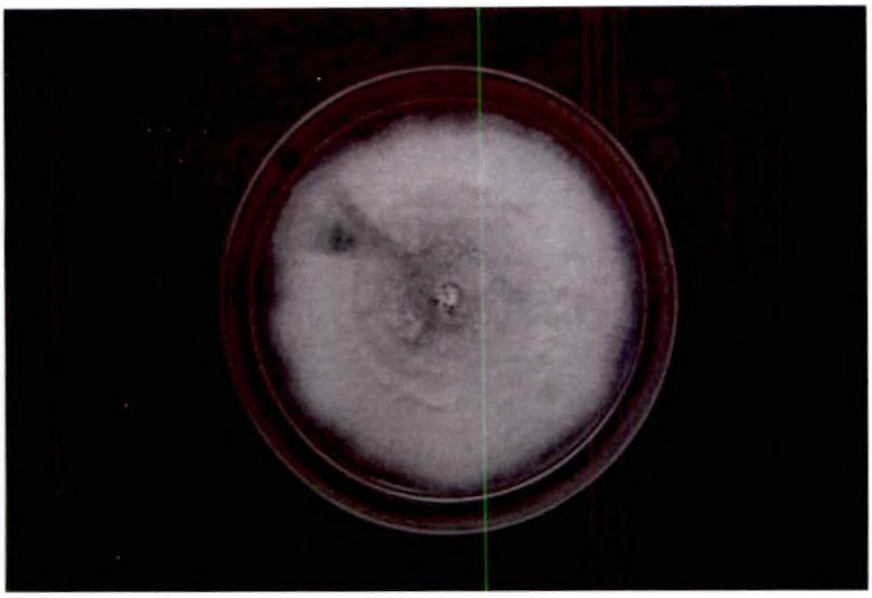


Plate 7: Culture of *Pestalotiopsis palmarum*

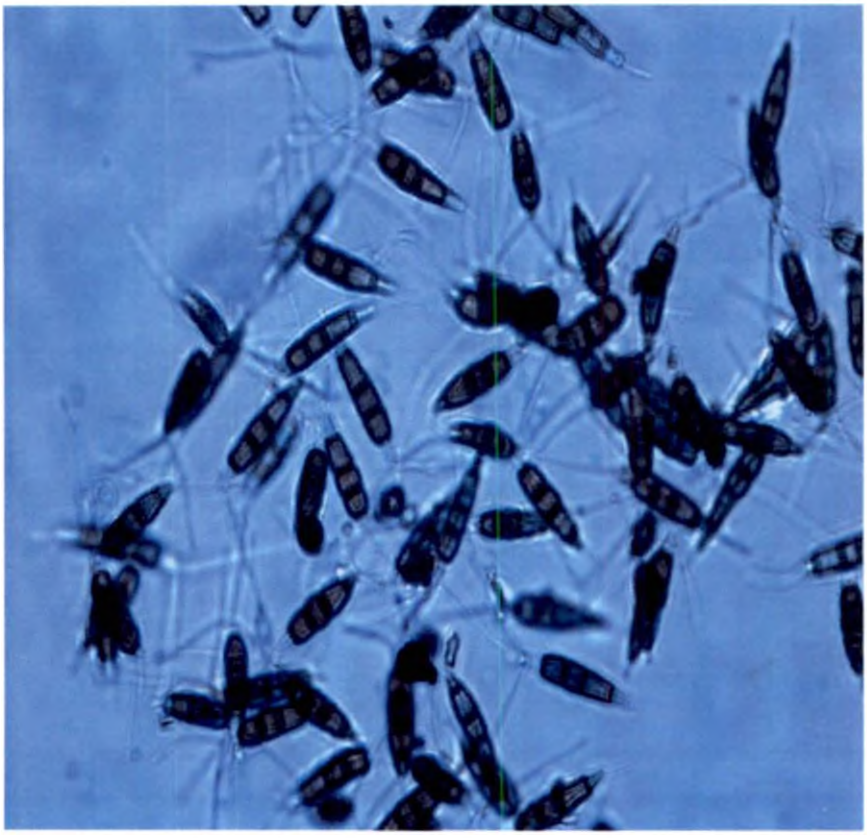


Plate 8: Conidia of *Pestalotiopsis palmarum*

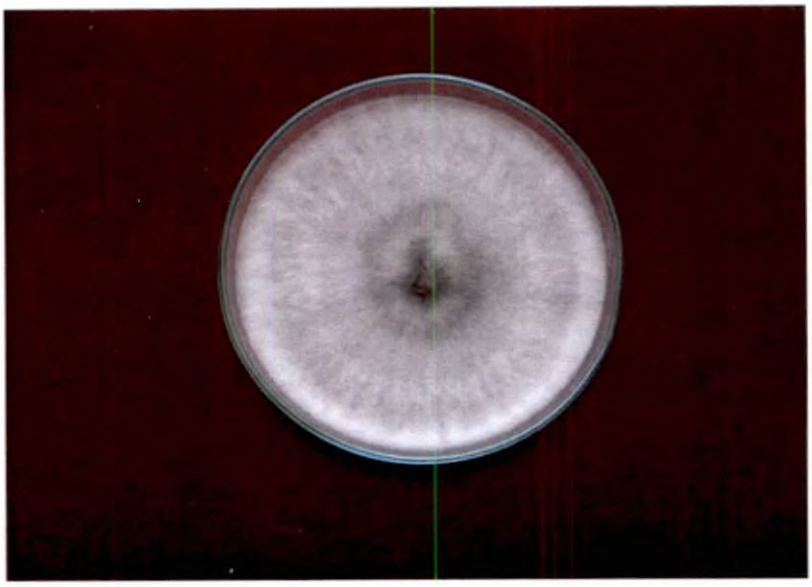


Plate 9: Culture of *Colletotrichum gloeosporioides*

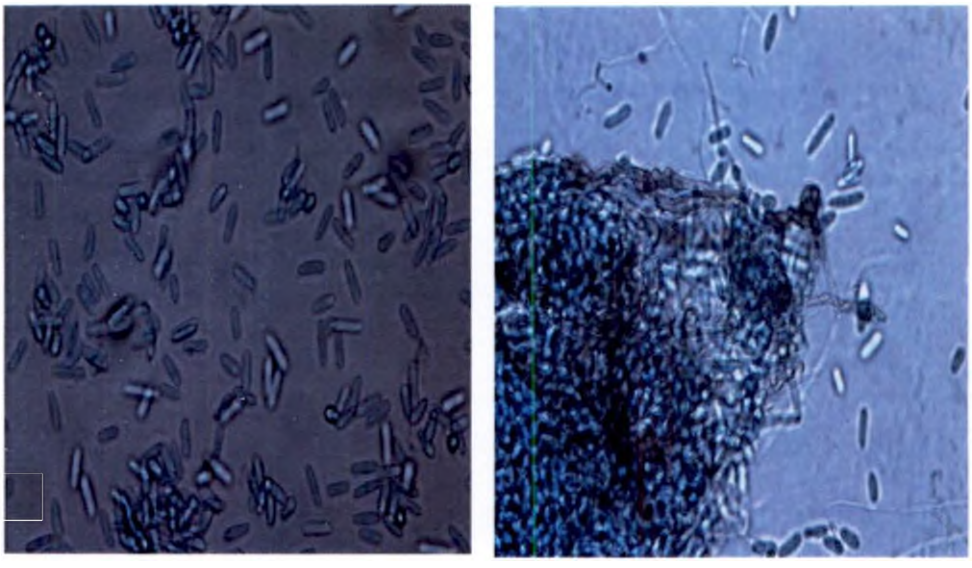


Plate 10: Conidia of *Colletotrichum gloeosporioides*

4.3.1.3 *Phomopsis palmicola*

The pycnidia observed in the straw coloured area were abundant in number and produced on the upper surface of the leaf. These were sub-erumpent, ostiolate, spherical to flatten and dark brown with thick wall of 5-6 layers and sometimes extending up to lower epidermis. The pycnidia were without stroma. Conidiophores were simple, short and 147.5-22.5 µm in length. Conidia were hyaline, single celled, uninuclear, which stained blue with cotton blue, elliptical with pointed ends (Plate 11).

The fungus produced white, thin to moderate mycelium with flat mycelial mats within the culture plate with even, margins. It took five days to cover the petri plates. It produced the pycnidia 8-10 days after inoculation; conidia were hyaline, elliptical with pointed ends and it was measuring 7.20 µm × 2.20 µm (Plate 12).

4.3.2 Molecular characterization of the leaf spot pathogens

All the three leaf spot pathogens isolates (Plate 13) viz., *Pestalotiopsis palmarum* (1, Vellayani), *Colletotrichum gloeosporioides* (CC1, CC2 from Balussery, Calicut, VC from Vellayani), *Phomopsis palmicola* (CP1, CP2 from Balussery, Calicut, PV from Vellayani) with their morphological and molecular identification based on ITS region and NCBI Gen bank accession numbers are given in Table 5. and the sequences obtained by Amplification of ITS region are presented in Table 6. All the leaf spot pathogens identified based on morphology were also confirmed by ITS identification in NCBI database.

The amplified products of DNA of *Pestalotiopsis palmarum, Colletotrichum gloeosporioides* and *Phomopsis palmicola* were approximately 650 bp in size (Fig 2).

The Cladogram (Fig 3.) obtained from Clustal omega database showed that sequences from CC1, CC2 and VC (*Colletotrichum gloeosporioides*) group together. Sequences from CC1, CC2 (from Calicut) were close and were related to the sequence from VC (from Vellayani). CP1, CP2 and PV formed another cluster (*Phomopsis palmicola*). The sequences from CP1, CP2 (Calicut) were close and were related to the sequences from PV (Vellayani). The sequence 1 (*Pestalotiopsis palmarum*, from Vellayani) was the outlier and most distant from the other organisms.

Multiple alignment of ITS region of *Pestalotiopsis palmarum*, *Colletotrichum gloeosporioides*, *Phomopsis palmicola* isolates showed, similarity between the isolates of v CP1, CP2 (*Phomopsis palmicola* from Balussery, Calicut) and they showed similarity to the

Plate 11: Culture of *Phomopsis palmicola*

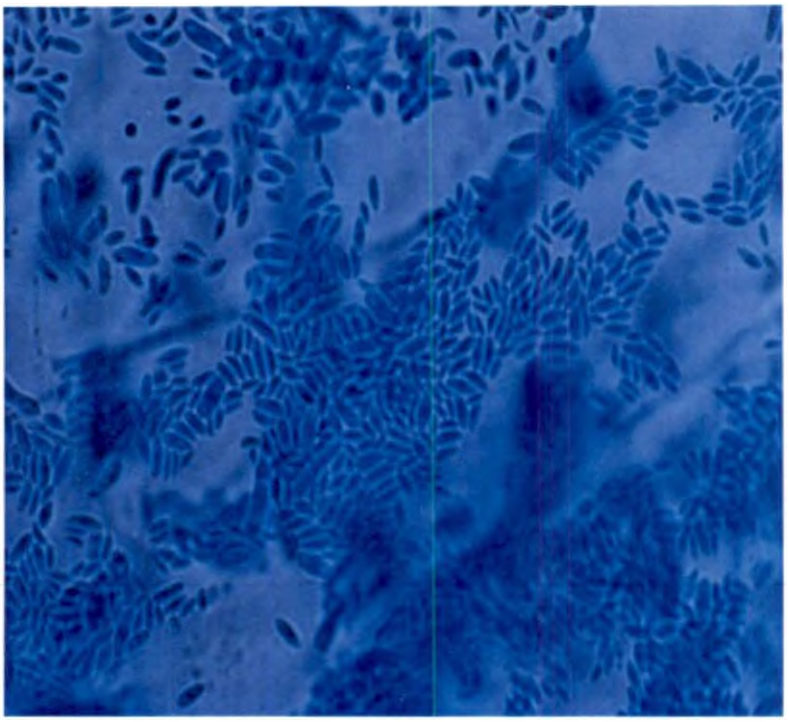
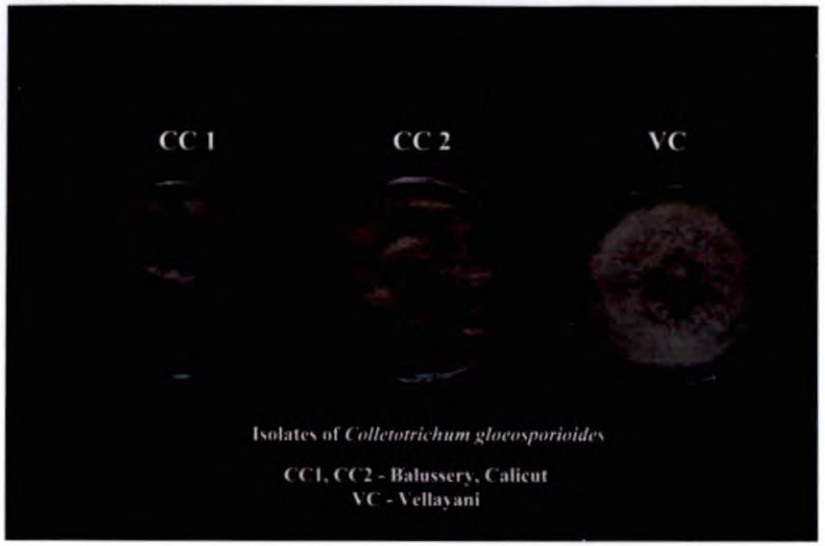


Plate 12: Conidia of *Phomopsis palmicola*



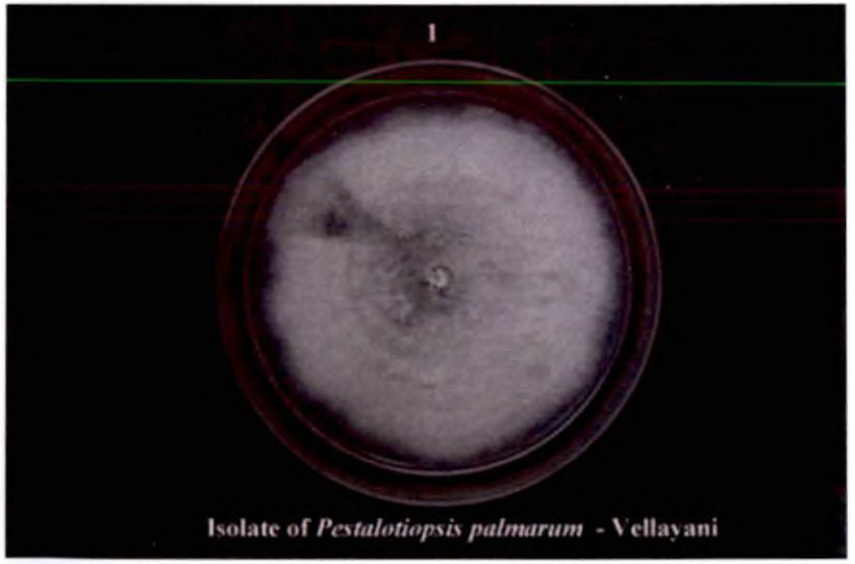


Fig 13: Isolates of Leaf spot pathogens of Arecanut

Table 5: Comparison of morphological and molecular identification of the leaf spot pathogens

S.I. No	Morphological identification	Place of collection	Mycelial characters		NCBI Gen Bank Accession number	Molecular identification (Based on ITS)	
			Above side of Petriplate	Under side of Petri plate		Per cent	Gen Bank accession no. with organism
1	*Colletotrichum gloeosporioides* - CC1	Calicut	Dull to greyish raised mycelium.	Greyish to dark black in colour	KF496900	100%	JN 390860.1-Colletotrichum sp. ITCC 6150
2	*Colletotrichum gloeosporioides* - CC2	Calicut	Dull greyish mycelium with Acervuli production	Black in colour	KF496901	100%	JQ 894656.1- *Colletotrichum sp* GM43A
3	*Colletotrichum gloeosporioides* - VC	Vellayani	Greyish mycelium	Black	KF496902	99%	AJ301907.1- *Colletotrichum gloeosporioides*
4	*Phomopsis palmicola*- CP1	Calicut	Dull to greyish mycelium with zonations	Black colour zonations	KF496903	99%	KC590096.1- *Phomopsis asparagi* ES-GDZS01
5	*Phomopsis palmicola*- CP2	Calicut	White coloured feathery mycelium, no zonations	White in colour	KF496904	99%	JF317194.1-*Phomopsis* sp. Mfer51
6	*Phomopsis palmicola* - PV	Vellayani	Dull grayish mycelium without zonations	Yellow in colour	KF496905	100%	GU066708.1-*Phomopsis* sp. 156 AH/T
7	*Pestalotiopsis palmarum* – 1	Calicut	Greyish white to black mycelium with Acervuli production	Yellowish black	KF481949	100%	JN651171.1-*Pestalotiopsis theae*

Table 6: Leaf spot pathogens with their ITS sequences

SI. No	Leaf spot pathogens with location of collection	Sequences of ITS region
1	*Colletotrichum gloeosporioides*-CC1 (Balussery , Calicut)	AGGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCC GGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTCAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTT GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGAC CCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCA GGTAGGAATACCCGCTGAACTTAA
2	*Colletotrichum gloeosporioides* - CC2 (Balussery , Calicut)	AGGGATCATTACTGAGTTTACGCTCTATAACCCTTTGTGAACATACCTATAACTGTTGCTTCGGCGGGTAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCC GGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTT GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACAGCTGATGTAGGCCCTCAAAGGTAGTGGCGGAC CCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCA GGTAGGAATACCCGCTGAACTTAA
3	*Colletotrichum gloeosporioides* -VC (Vellayani)	AGGGATCATTACTGAGTTTACGCTCTACAACCCTTTGTGAACATACCTACAACTGTTGCTTCGGCGGGCAGGGTCTCCGTGACCCTCCCGGCCTCCCGCCCCC GGGCGGGTCGGCGCCCGCCGGAGGATAACCAAACTCTGATTTAACGACGTTTCTTCTGAGTGGTACAAGCAAATAATCAAAACTTTTAACAACGGATCTCTT GGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAG CATTCTGGCGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCCCTACGGCTGACGTAGGCCCTCAAAGGTAGTGGCGGAC CCTCCCGGAGCCTCCTTTGCGTAGTAACTTTACGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAACCCCCAATTTTCCAAAGGTTGACCTCGGATCA GGTAGGAATACCCGCTGAACTTAA
4	*Phomopsis palmicola* - CP1 (Balussery, Calicut)	AGGGATCATTGCTGGAACGCGCCCCAGGCGCACCCAGAAACCCTTTGTGAACTTATACCTTACTGTTGCCTCGGCGCATGCCGGCCCCCCCGGGGGCCCCTC GGAGACGAGGAGCAGGCACGCCGGCGGCCAAGTTAACTCTTGTTTTTACACTGAAACTCTGAGAAAAAACACAAATGAATCAAAACTTTCAACAACGGATC TCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTC TGGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACTGCTTGGTGTTGGGGCACTGCTTTTTACCCAAGAAGCAGGCCCTGAAATCTA GTGGCGAGCTCGCCAGGACCCCGAGCGCAGTAGTTAAACCCTCGCTCTGGAAGGCCCTGGCGGTGCCCTGCCGTTAAACCCCCAACTCTTGAAAATTTGACC TCGGATCAGGTAGGAATACCCGCTGAACTTAA
5	*Phomopsis palmicola* -CP2 (Balussery, Calicut)	AGGGATCATTGCTGGAACGCGCCCCAGGCGCACCCAGAAACCCTTTGTGAACTTATACCTTACTGTTGCCTCGGCGCATGCTGGCCCCCTCGGGGTCCCTTG GAGACAAGGAGCAGGCACGCCGGCGGCCAAGTTAACTCTTGTTTTTACACTGAAACTCTGAGAAAAAACACAAATGAATCAAAACTTTCAACAACGGATCT CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT GGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCATTGCTTGGTGTTGGGGCACTGCTTTTTACCCAAAAAGCAGGCCCTGAAATCTAG TGGCGAGCTCGCCAGGACCCCGAGCGCAGTAGTTAAACCCTCGCTTTGGAAGGCCCTGGCGGTGCCCTGCCGTTAAACCCCCAACTTTTGAAAATTTGACCT CGGATCAGGTAGGAATACCCGCTGAACTTAA
6	*Phomopsis palmicola* – PV (Vellayani)	AGGGATCATTGCTGGAACGCGCCCCAGGCGCACCCAGAAACCCTTTGTGAACTTATACCTTTTGTTGCCTCGGCGCATGCTGGCCTCTAGTAGGCCCCTCACC CCGGTGAGGAGAAGGCACGCCGGCGGCCAAGTTAACTCTTGTTTTTACACTGAAACTCTGAGAAAAAACACAAATGAATCAAAACTTTCAACAACGGATCT CTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTCT GGTATTCCGGAGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCACTGCTTGGTGTTGGGGCACTGCTTTAAACGGAGCAGGCCCTGAAATCTAGTGG CGAGCTCGCCAGGACCCCGAGCGCAGTAGTTAAACCCTCGCTCTGGAAGGCCCTGGCGGTGCCCTGCCGTTAAACCCCCATCTTTTGAAAATTTGACCTCGG ATCAGGTAGGAATACCCGCTGAACTTAA
7	*Pestalotiopsis palmarum* -1 (Balussery, Calicut)	AGGGATCATTATAGAGTTTTCTAAACTCCCAACCCATGTGAACTTACCTTTTGTTGCCTCGGCAGAGGTTACCTGGTACCTGGAGACAGGTTACCCTGTAGCA GCTGCCGGTGGACTACTAAACTCTTGTTATTTTATGTAATCTGAGCGTCTTATTTTAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATG AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCATGC CTGTTCGAGCGTCATTTCAACCCTTAAGCCTAGCTTAGTGTTGGGAATTTACAGTTATGTAATTCCTGAAATACAACGGCGGATCTGTGGTATCCTCTGAGCG TAGTAAATTATTTCTCGCTTTTGTCAGGTGCTGCAGCTCCCAGCCGCTAAACCCCCAATTTTTTGTGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACT TAA

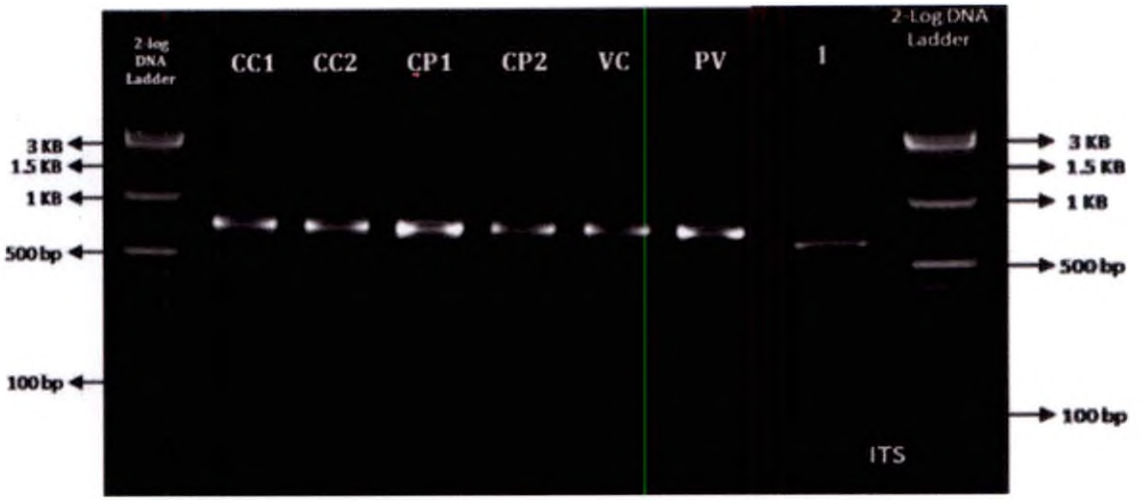


Fig 2. ITS region Amplification of leaf spot pathogens, viz., *Colletotrichum gloeosporioides* (CC1, CC2, VC), *Phomopsis palmicola (*CP1, CP2, PV), and *Pestalotiopsis palmarum* (1).

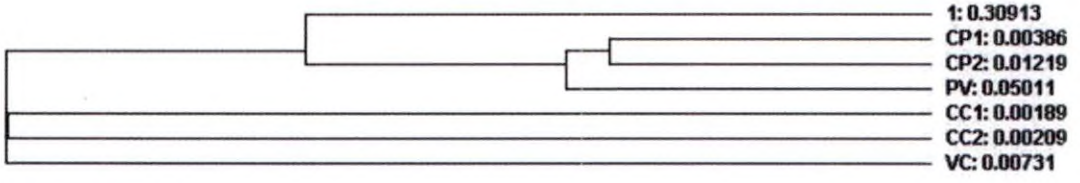


Fig 3. Cladrogram of isolates of leaf spot pathogens with distances between them (1- *Pestalotiopsis palmarum*, CP1, CP2, PV - *Phomopsis palmicola*, CC1, CC2, VC - *Colletotrichum gloeosporioides*)

isolate PV (*Phomopsis palmicola* from Vellayani). The isolates CC1 and CC2 (*Colletotrichum gloeosporioides* from Balussery, Calicut) showed sequence similarity to each other and the in turn were similar to VC (*Colletotrichum gloeosporioides* from Vellayani). The sequence of isolate 1 (*Pestalotiopsis palmarum* from Vellayani) showed that it was most dissimilar to the sequences from all other isolates. (Fig 4).

4.3.3 Growth of *C. gloeosporioides* on different solid media

The results from the Table 5 revealed that the fungus recorded full growth (90.00 mm) on PDA, Czapek's agar, Richard's agar and Sabouraud's agar, they were on par with Corn meal agar (89.30 mm). The least growth of the fungus was observed in Malt extract agar (72.10 mm). Plate 14, Fig 5.

4.3.4 Effect of pH on the growth of the fungus

4.3.4.1 Effect of pH on the growth of *P. palmarum*

Data from the Table 8 revealed that, *P. palmarum* first completed its growth at pH 5.0 (90 mm) on the seventh day of its inoculation and it was significantly superior over other pH levels. Followed by pH 6.0 (86.60 mm), 6.5 (86.27 mm) and pH 7.0 (85.80 mm), they were on par with each other. The least growth was observed at pH 8.0 (65.70 mm). Plate 15, Fig 6.

4.3.4.2 Effect of pH on the growth of *C. gloeosporioides*

Data from the (Table 9) revealed that the test fungus first completed its growth at pH 5.0 (90 mm) on the sixth day of its inoculation and the growth was significantly superior over all other pH levels tested. This was followed by growth at pH 6.0 (86.7 mm), 6.5 (85.7 mm) and 7.0 (83.9 mm) and these were on par with each other. The least growth of the fungus (64.5 mm) was observed at pH 8.0 (Plate 16, Fig 7).

```
CP1             AGGGATCATT GCTGGAACGC GCCCCAGGCG CACCCAGAAA CCCTTTGTGA
CP2             AGGGATCATT GCTGGAACGC GCCCCAGGCG CACCCAGAAA CCCTTTGTGA
PV              AGGGATCATT GCTGGAACGC GCCCCAGGCG CACCCAGAAA CCCTTTGTGA
CC1             AGGGATCATT ACTGAGTT.. ........TA CGCTCTACAA CCCTTTGTGA
CC2             AGGGATCATT ACTGAGTT.. ........TA CGCTCTATAA CCCTTTGTGA
VC              AGGGATCATT ACTGAGTT.. ........TA CGCTCTACAA CCCTTTGTGA
1               AGGGATCATT ATAGAGTTT. ........TC TAAACTCCCA ACCCATGTGA

CP1             ACTTATACCT TACTGTTGCC TCGGCG..CA TG....CCG. GCCCCCCCGG
CP2             ACTTATACCT TACTGTTGCC TCGGCG..CA TG....CTG. GCCCCCTCGG
PV              ACTTATACCT TT.TGTTGCC TCGGCG..CA TG....CTG. GCCTCTAGTA
CC1             ACATACCTAT AACTGTTGCT TCGGCGGGTA GGGTCTCCGT GACCCTCCCG
CC2             ACATACCTAT AACTGTTGCT TCGGCGGGTA GGGTCTCCGT GACCCTCCCG
VC              ACATACCTAC AACTGTTGCT TCGGCGGGCA GGGTCTCCGT GACCCTCCCG
1               ACTTACCTTT TG...TTGCC TCGGCAG..A GGTTACCTG. GTACCTGGAG

CP1             GGGCCCCTCG G..AGACGAG GAGCAGGCAC GCCGGCGGCC AAGTTAACTC
CP2             GG.TCCCTTG G..AGACAAG GAGCAGGCAC GCCGGCGGCC AAGTTAACTC
PV              GG.CCCCTCA CCCCGGTGAG GAGAAGGCAC GCCGGCGGCC AAGTTAACTC
CC1             GCCTCCCGCC CCCGGGCGGG TCGGCG.CCC GCCGGAGGAT AACCAAACTC
CC2             GCCTCCCGCC CCCGGGCGGG TCGGCG.CCC GCCGGAGGAT AACCAAACTC
VC              GCCTCCCGCC CCCGGGCGGG TCGGCG.CCC GCCGGAGGAT AACCAAACTC
1               ACAGGTTACC C.......TG TAGCAG..CT GCCGGTGGAC TACTAAACTC

CP1             TTGTTTTTAC ACTGAAACTC TGAGAAAAAA CACAAATGAA TCAAAACTTT
CP2             TTGTTTTTAC ACTGAAACTC TGAGAAAAAA CACAAATGAA TCAAAACTTT
PV              TTGTTTTTAC ACTGAAACTC TGAGAAAAAA CACAAATGAA TCAAAACTTT
CC1             TGATTCAACG ACGTTTCTTC TGAGTGGTAC AAGCAAATAA TCAAAACTTT
CC2             TGATTTAACG ACGTTTCTTC TGAGTGGTAC AAGCAAATAA TCAAAACTTT
VC              TGATTTAACG ACGTTTCTTC TGAGTGGTAC AAGCAAATAA TCAAAACTTT
1               TTGTTATTTT ATGTAATCTG AGCGTCTTAT TT.TAATAAG TCAAAACTTT

CP1             CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC
CP2             CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC
PV              CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC
CC1             TAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC
CC2             TAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC
VC              TAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC
1               CAACAACGGA TCTCTTGGTT CTGGCATCGA TGAAGAACGC AGCGAAATGC

CP1             GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
CP2             GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
PV              GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
CC1             GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
CC2             GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
VC              GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG
1               GATAAGTAAT GTGAATTGCA GAATTCAGTG AATCATCGAA TCTTTGAACG

CP1             CACATTGCGC CCTCTGGTAT TCCGGAGGGC ATGCCTGTTC GAGCGTCATT
CP2             CACATTGCGC CCTCTGGTAT TCCGGAGGGC ATGCCTGTTC GAGCGTCATT
PV              CACATTGCGC CCTCTGGTAT TCCGGAGGGC ATGCCTGTTC GAGCGTCATT
CC1             CACATTGCGC CCGCCAGCAT TCTGGCGGGC ATGCCTGTTC GAGCGTCATT
CC2             CACATTGCGC CCGCCAGCAT TCTGGCGGGC ATGCCTGTTC GAGCGTCATT
VC              CACATTGCGC CCGCCAGCAT TCTGGCGGGC ATGCCTGTTC GAGCGTCATT
1               CACATTGCGC CCATTAGTAT TCTAGTGGGC ATGCCTGTTC GAGCGTCATT
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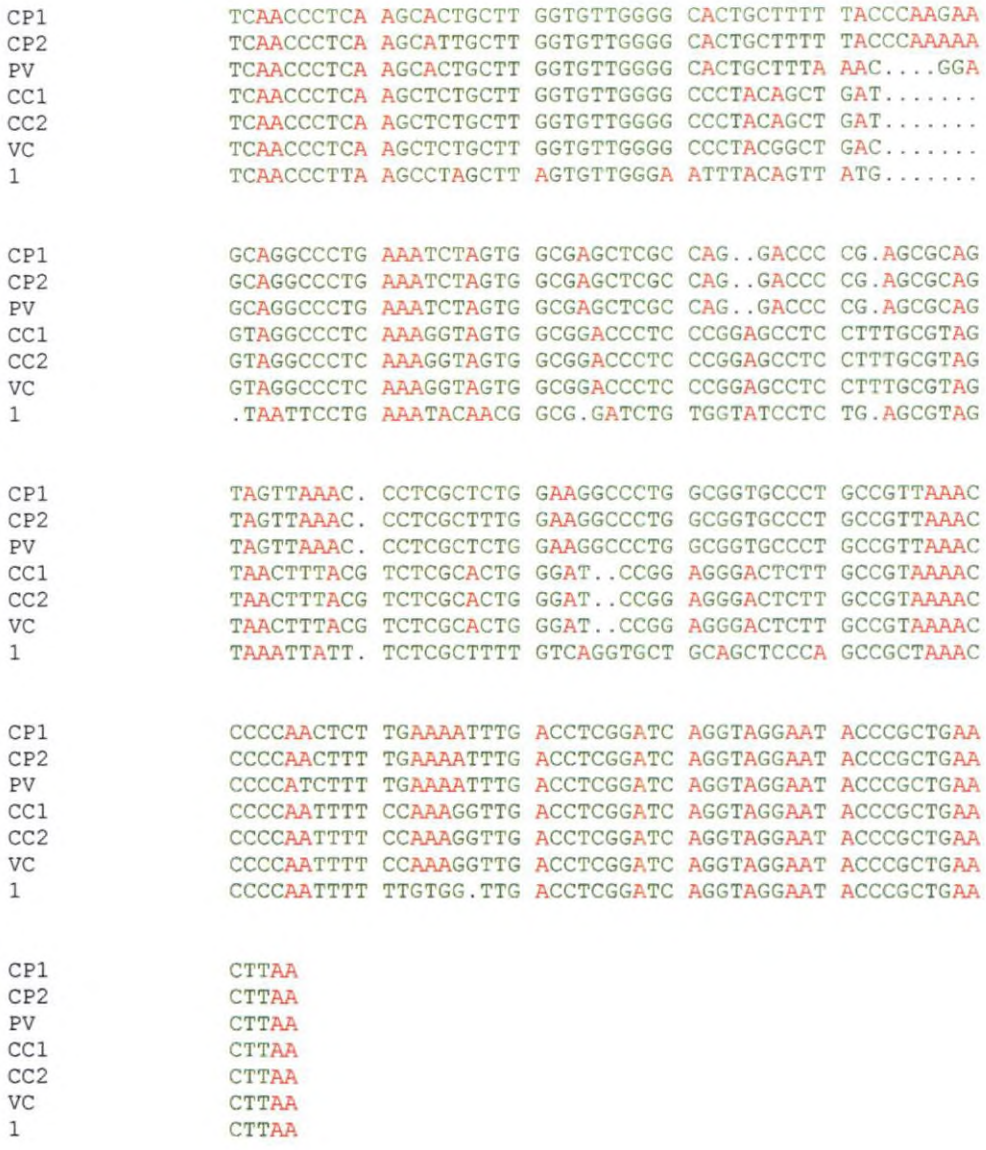


Fig 4 : Multiple alignment of ITS region of leaf spot pathogens - *Phomopsis palmicola* (CP1, CP2, PV), *Colletotrichum gloeosporioides*(CC1,CC2,VC), *Pestalotiopsis palmarum* (1) isolates.

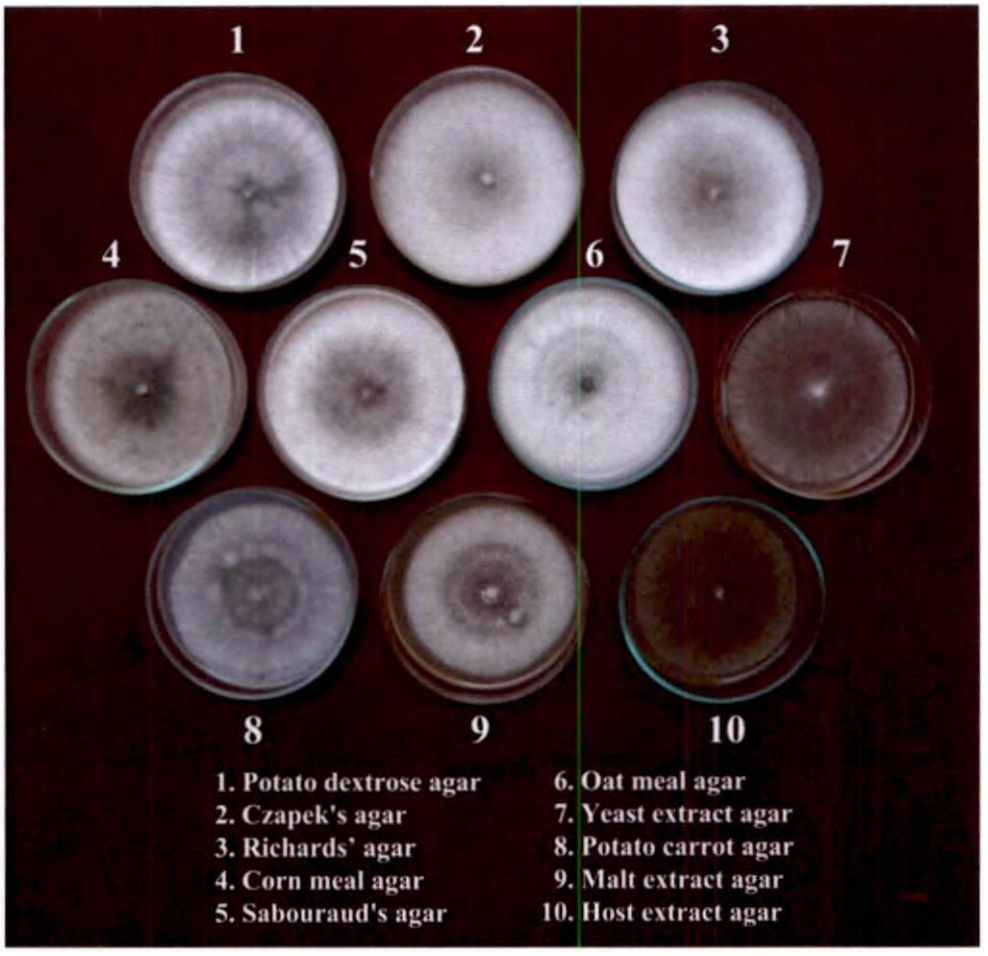


Plate 14. Growth of *Colletotrichum gloeosporioides* on different solid media

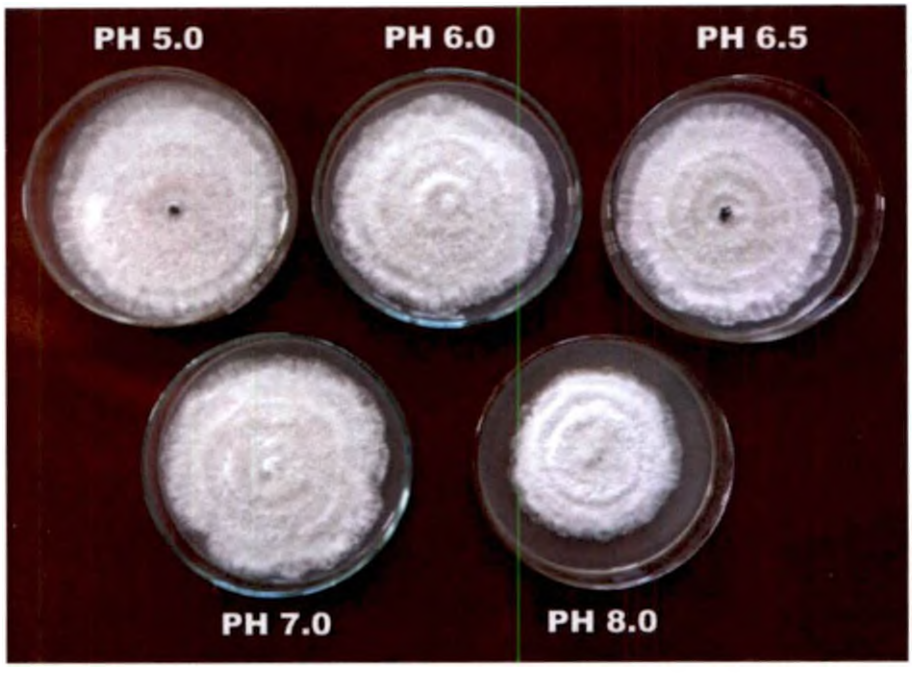


Plate 15: Effect of pH on the growth of *Pestalotiopsis palmarum*

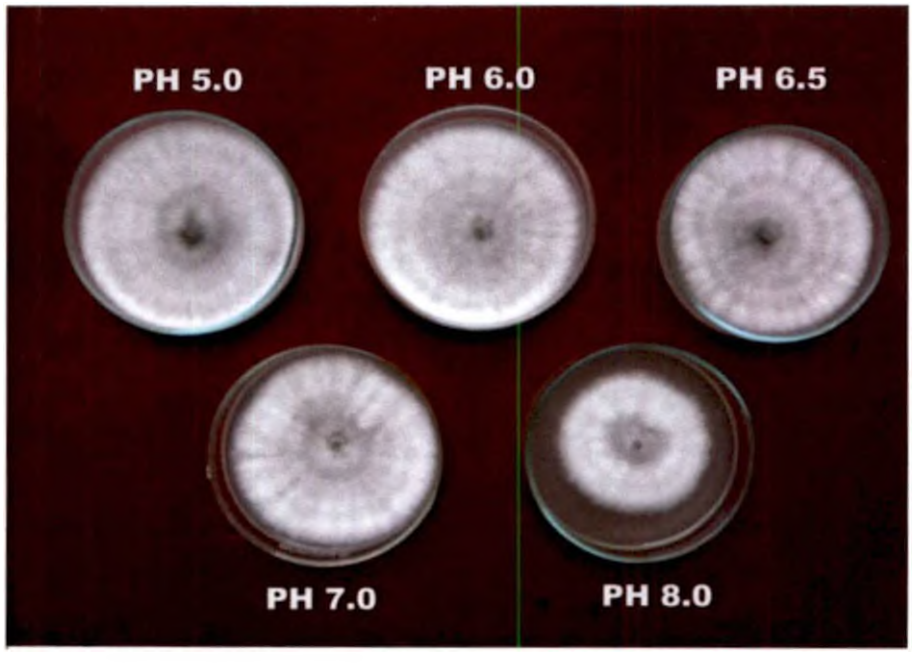


Plate 16: Effect of pH on the growth of *Colletotrichum gloeosporioides*

Table 7: Growth of *Colletotrichum gloeosporioides* on different solid media

SI. No	Different Media	Mycelial characters		
		Radial growth (mm)	Colour	Type of growth
1	Potato dextrose agar	90*	Intermixed black and white	Flat growth circular
2	Czapek's agar	90	White	Fluffy
3	Richard's agar	90	White	Fluffy, raised irregular
4	Corn meal agar	89.3	Greyish to black in colour	Flat growth circular
5	Sabouraud's agar	90	Milky white to black	Raised, fluffy growth
6	Oat meal agar	73.6	Milky white	Dense even growth with circular margins
7	Yeast extract agar	76	Dull white	Thin mycelium
8	Potato carrot agar	77.33	Light white and grey	Cottony growth
9	Malt extract agar	72.1	White	Even, circular
10	Host extract agar	73.5	Dull white	Sparse, circular
C.D (0.05 level)		0.23		

* Mean of three replications

Table 8: Effect of pH on the growth of *Pestalotiopsis palmarum*

SI. No	pH level	Radial growth (mm)
1	5.0	90*
2	6.0	86.60
3	6.5	86.20
4	7.0	85.80
5	8.0	65.70
	C.D (0.05 level)	0.37

* Mean of five replications

Table 9 : Effect of pH on the growth of *Colletotrichum gloeosporioides*

SI. No	pH level	Radial growth (mm)
1	5.0	90*
2	6.0	86.7
3	6.5	85.7
4	7.0	83.9
5	8.0	64.5
	C.D (0.05 level)	0.38

* Mean of five replications

Fig 5: Growth of *Colletotrichum gloeosporioides* on different solid media

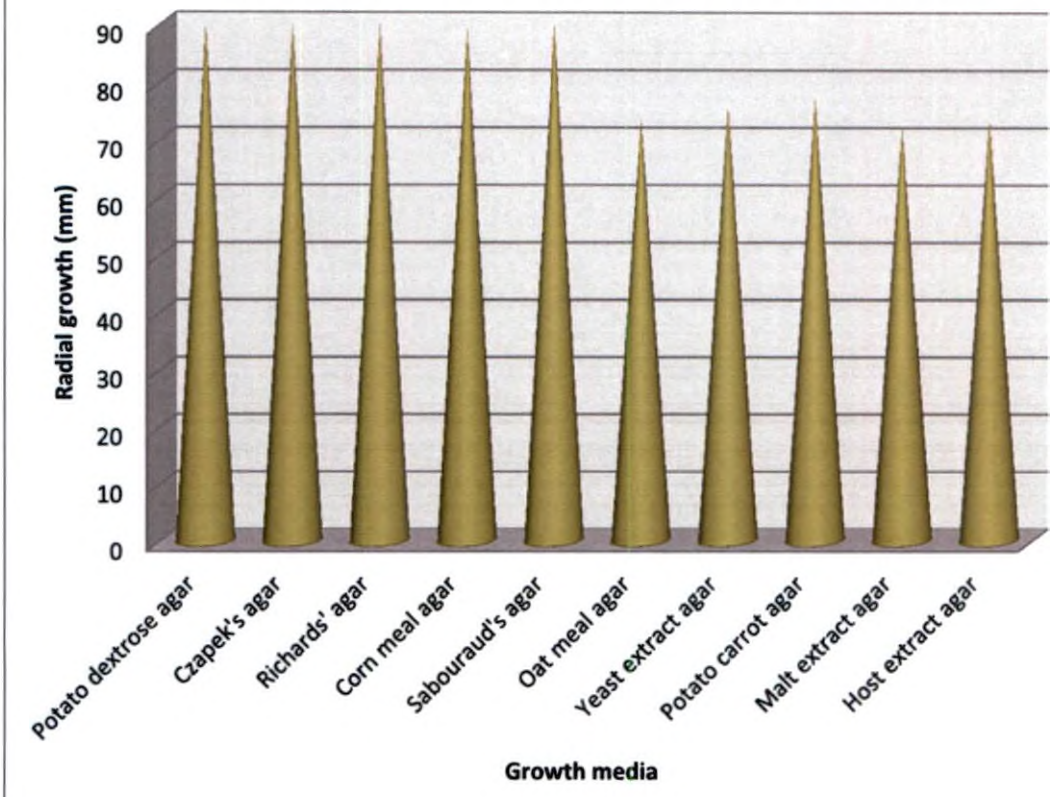


Fig 6 : Effect of pH on the growth of *Pestalotiopsis palmarum*

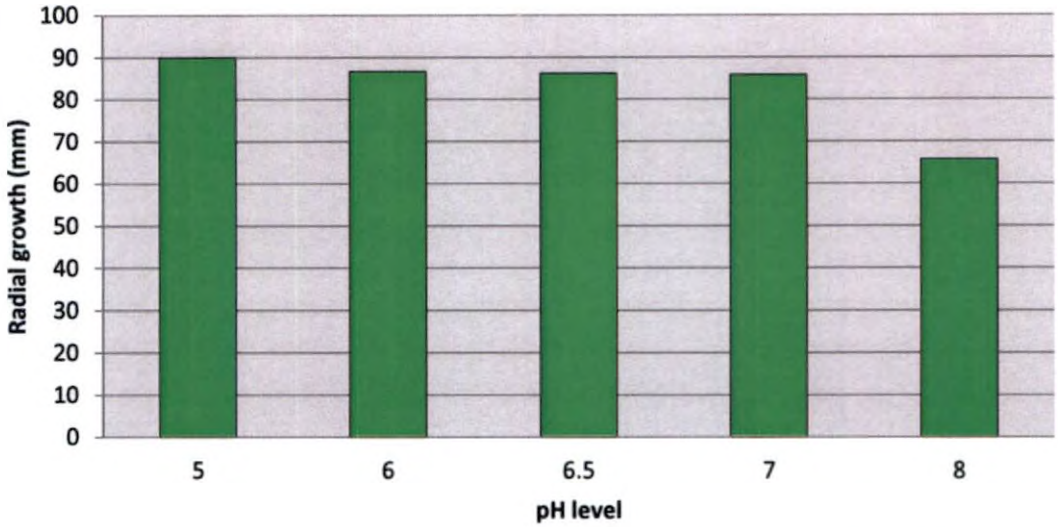
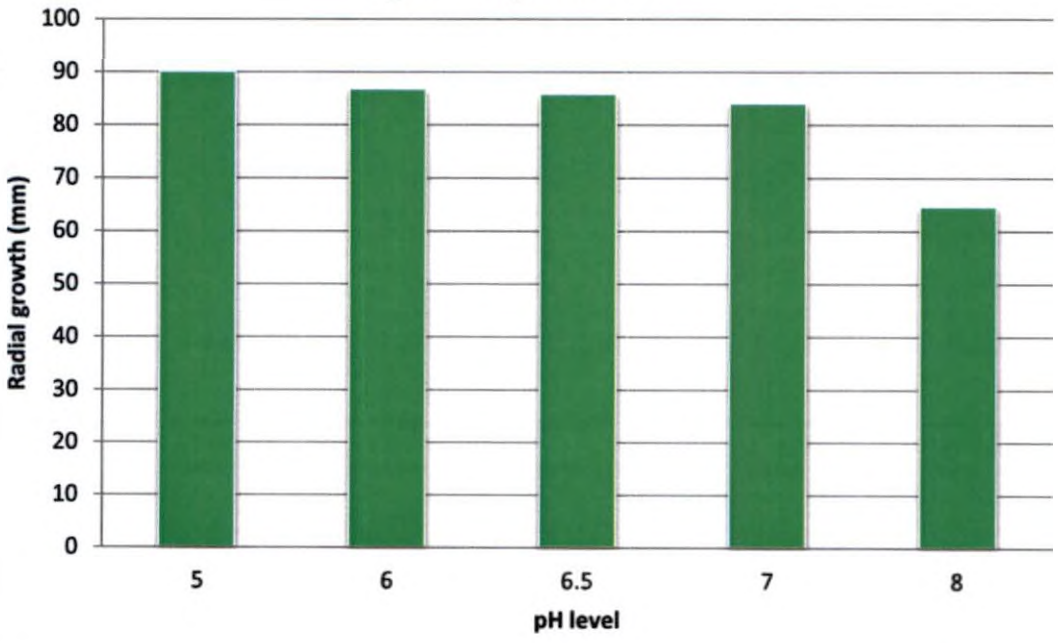


Fig 7: Effect of pH levels on *Colletotrichum gloeosporioides*



4.4 Survey on the incidence and intensity of leaf spots

The leaf spots caused by *P. palmarum*, *C. gloeosporioides* and *P. palmicola* were observed on different plants and also in same plant with different symptoms.

On young areca palms below 10 years, grown in I.F. Vellayani, leaf spots caused by *P. palmarum* and *C. gloeosporioides* were prominent. A survey was undertaken to study the incidence and intensity. Survey on disease incidence was also done in Balussery and Peringamala.

The survey revealed that per cent disease incidence was maximum in Balussery (85.0), followed by Peringamala (83.33) and I.F. Vellayani (75.0). In the I.F. Vellayani the disease index was 34.5 % (Table 10).

The results of the survey also indicated that the first three leaves from top were free from leaf spot diseases. The maximum disease (Per cent Disease Index (PDI) was recorded on eighth leaf (38.51) followed by seventh (27.68), sixth (18.72), fifth (10.80) and fourth leaf (7.63) (Table 11).

4.5 Effect of weather factors on the incidence and intensity of leaf spot diseases

The weather data was collected from the Meteorological Dept. of College of Agriculture, Vellayani for the period from April 2012 to March 2013. The survey on leaf spot disease was conducted on 29th of each month on the young areca palm of Mangala variety in the I.F. Vellayani.

Based on the disease score chart, all the leaves of the selected plant were scored individually. Average PDI of a month was calculated by summing the PDI of individual six plants together. The results revealed (Table 12) (Fig. 8) that the disease occurred all round the year and PDI ranged from 20.13 to 33.21 for different months. It was observed that disease gradually increased from June to November from 21.90 to 33.21 %. During this period many fresh leaf spots were formed. After November the disease was gradually decreasing up to May. Formation of fresh infections declined during the same period.

The maximum disease intensity of 33.21 was observed in November when weather recorded maximum temperature (30.2°C), minimum temperature (23°C), relative humidity (96.4%) and rainfall (111.1 mm). Lowest intensity was recorded during May, when the

Table 10: Disease incidence and Disease index of arecanut leaf spot disease in different locations.

SI.No	Location	No. plants observed	No. of plants diseased	Disease incidence (%)	Disease Index (%)
1	I.F. Vellayani	80	60	75	34.5 *
2	Peringamala Thiruvananthapuram	30	25	83.33	-
3	Balussery Calicut	100	85	85	-

* Mean of 80 plants

Table 11: Intensity of leaf spots as influenced by position of leaves on arecanut palm

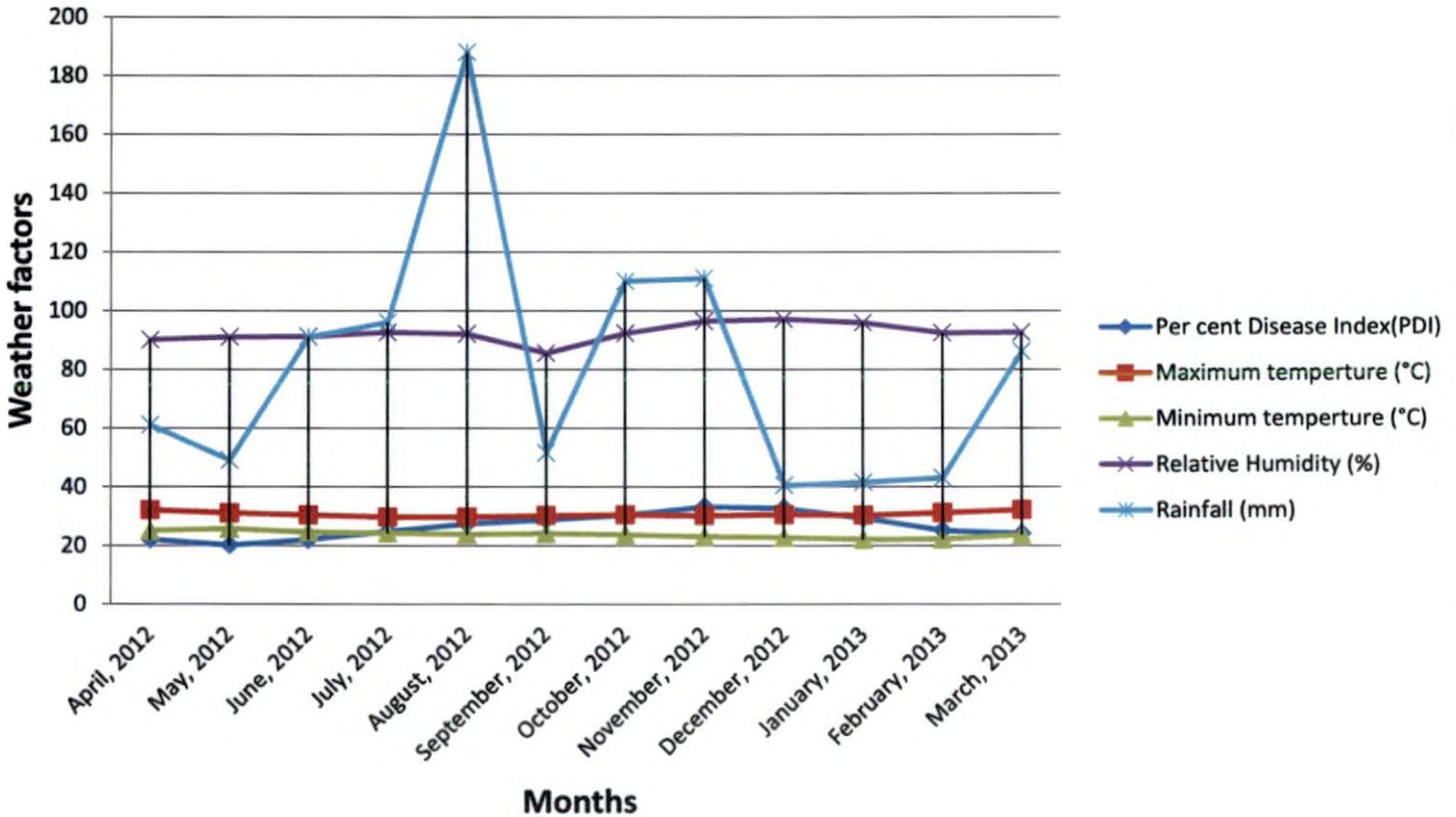
Position of leaf from top	Disease Index (%)
First	0.00 *
Second	0.00
Third	0.00
Fourth	7.63
Fifth	10.80
Sixth	18.72
Seventh	27.68
Eighth	38.51

* Mean of 80 plants

Table 12: Disease Index of arecanut leaf spots and weather data at Instructional Farm, Vellayani

Months	Per cent Disease Index (PDI)	Weather factors (monthly means)			
		Temperature (^{0}C)		Relative Humidity (%)	Rainfall (mm)
		Maximum	Minimum		
April, 2012	21.96	32.1	25	90	60.9
May, 2012	20.13	31.1	25.7	91	49
June, 2012	21.90	30.4	24.5	91.2	91
July, 2012	24.69	29.7	24.2	92.6	96
August, 2012	27.31	29.7	23.8	92	188
September, 2012	28.79	30.2	24.1	85.5	51.5
October, 2012	30.41	30.5	23.7	92.2	110
November, 2012	33.21	30.2	23	96.4	111.1
December, 2012	32.72	30.6	22.7	97.1	40.5
January, 2013	29.30	30.3	22	95.9	41.5
February, 2013	25.12	31.2	22.1	92.4	43
March, 2013	24.07	32.2	23.7	92.7	86

Fig 8: Influence of weather data and Disease Index of arecanut leaf spot at Instructional Farm, Vellayani



weather data showed maximum temperature (32.1° C) and minimum temperature (25.7°C), relative humidity (91 %) and rainfall (49 mm).

Correlation was worked out with weather data and PDI. The results are presented in the Table 11. It was observed that maximum temperature and minimum temperature were negatively correlated and relative humidity and rainfall were positively correlated with PDI.

But, highly significant negative correlation was observed between minimum temperature (-0.6725) and PDI. This means that as the minimum temperature increases the PDI decreases and vice-versa. All other weather parameters were non-significant

4.6.1 Isolation of antagonistic organisms from the phyllosphere of healthy areca palms

Two fungi and two bacteria were obtained from the phyllosphere on repeated isolation. The two fungi were identified as *Aspergillus* sp. and *Penicillium* sp. The two bacteria were noted as B_1 and B_2 (Table 14).

4.6.2 Isolation of antagonists from rhizosphere of healthy areca palms

From the rhizosphere, three fungi were isolated and one fungus was identified as *Trichoderma harzianum*. The others were an yellow ascomycete fungus and a greyish white fungus (Table 14).

4.7 *In vitro* studies

4.7.1.1 *In vitro* evaluation of bioagents against *P. palmarum*

The results (Table 15, Plate 17) revealed that *Trichoderma harzianum* was antagonistic to *Pestalotiopsis palmarum* with (62.77 %) inhibition, followed by bacteria B1 showing (40.33 %) inhibition on the test fungus.

4.7.1.2 *In vitro* evaluation of bioagents against *C. gloeosporioides*

Results (Table 16, Plate 18) showed that, *T. harzianum* inhibited 51.04 % growth of *C. gloeosporioides.* Its antagonistic effect was significantly superior over all other antagonists tried, followed by bacteria B1 with 46.72 % inhibition.

Table 13: Correlation between Per cent Disease Index and weather parameters

SI. No	Weather parameters	Correlation Co-efficient 'r' values
1.	Maximum Temperature	-0.4568
2.	Minimum Temperature	-0.6725*
3.	Rainfall	0.4922
4.	Relative Humidity	0.1142

* Highly Significant at 0.05 level of probability

Table 14: Fungal and Bacterial antagonists isolated from Phyllosphere and Rhizosphere of arecanut palms

Plant part	Organisms isolated
Phyllosphere	1.*Aspergillus* sp
	2.*Penicillium* sp
	3.Bacteria (B1)
	4.Bacteria (B2)
Rhizosphere	1.*Trichoderma harzianum*
	2.An yellow ascomycete fungus
	3.A greyish white fungus

Table 15: Antagonistic activity of bioagents against *Pestalotiopsis palmarum*

SI.No	Bioagents	Growth inhibition (%) on *P. palmarum*
1.	*Trichoderma harzianum*	62.77 *
2.	*Aspergillus sp*	0
3.	*Penicillium sp*	0
4.	An yellow ascomycete fungus	0
5.	A whitish grey fungus	0
6.	Bacteria (B1)	40.33
7.	Bacteria (B2)	29.6
C.D (0.05 level)		1.37

*Mean of three replications

Table 16: Antagonistic activity of bioagents against *Colletotrichum gloeosporioides*

SI.No	Bioagents	Growth inhibition on *C. gloeosporioides* (%)
1.	*Trichoderma harzianum*	51.04*
2.	*Aspergillus sp*	0
3.	*Penicillium* sp	0
4.	An yellow ascomycete fungus	0
5.	A greyish white fungus	0
6.	Bacteria (B1)	46.72
7.	Bacteria (B2)	40.2
C.D (0.05 level)		2.46

*Mean of three replications

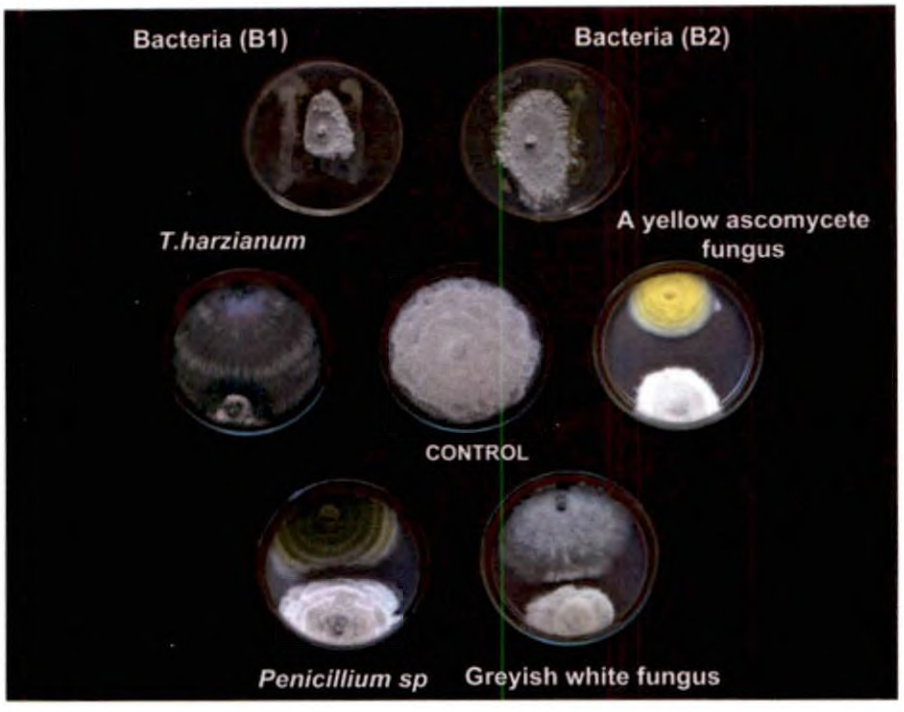


Plate 17: Antagonistic activity of bioagents against *Pestalotiopsis palmarum*

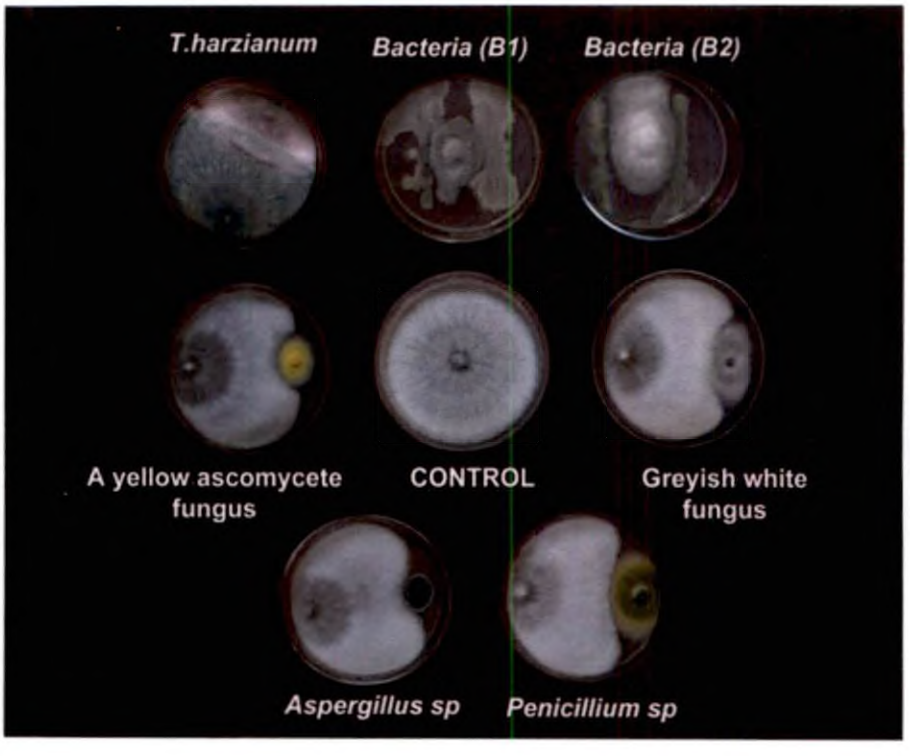


Plate 18: Antagonistic activity of bioagents against *Colletotrichum gloeosporioides*

4.7.2.1 *In vitro* evaluation of botanicals against *P. palmarum*

Five plant extracts viz., Mari gold, Bougainvillea, Datura at 10, 20 and 30 %, Garlic and Neem at 2, 4 and 6 % were evaluated to study their effectiveness against *P. Palmarum.* Data is presented in Table 17and depicted in Fig 9.

Out of the five botanicals tested, Garlic bulb extract at two per cent concentration was found to be the best in inhibiting the mycelial growth of *P.palmarum* (82.26 %) and found superior over all the other extracts, followed by Datura (51.23 %), Bougainvillea (33.37 %), Neem (15.58 %) and Mari gold (9.81 %) which were significantly different in their effectiveness.

Out of the five botanicals tested, Garlic bulb extract at four per cent gave maximum inhibition of the fungus (84.50 %), followed by Datura (55.39 %) and Bougainvillea (43.56 %). The least inhibition was found in Neem (24.43 %) and Mari gold (11.16 %). All the botanicals in this dose were significantly different in their effectiveness against *P. palmarum*

Out of the five botanicals tested, Garlic at six per cent gave (88.54 %) inhibition on the fungus followed by Bougainvillea (60.56 %) and Datura (57.81 %) and they remain on par with each other. Least inhibition was found in Mari gold (17.71 %).

Results of this experiment revealed that Garlic bulb extract was highly effective in inhibiting the mycelial growth of the fungus compared to all other extracts and the least inhibition was observed with Neem and Mari gold leaf extracts (Plate 19).

4.7.2.2 *In vitro* evaluation of botanicals against *C. gloeosporioides*

Five plant extracts viz., Mari gold, Bougainvillea, Datura at 10, 20 and 30 %, Garlic and Neem at 2, 4 and 6 % were evaluated to study their effectiveness against *C. gloeosporioides.* Data are presented in Table 18 and Fig 10. The results revealed that, the efficacy of plant extracts at different concentrations on per cent inhibition of mycelial growth of *C. gloeosporioides* differed significantly.

Table 17: Effect of botanicals on mycelial growth inhibition of *Pestalotiopsis palmarum*

SI. No	Botanicals	Mycelial growth inhibition over control (%)		
		Concentrations (%)		
		10	20	30
1	*Tagetes erecta* (leaf) Mari gold	9.81* (18.24)#	11.16 (19.51)	17.71 (24.88)
2.	*Bougainvillea spectabilis* (leaf) Bougainvillea	33.37 (35.27)	43.56 (41.28)	60.56 (51.08)
3.	*Datura stramonium* (leaf) Datura	51.23 (45.68)	55.39 (48.07)	57.81 (49.97)
		2	4	6
4.	*Allium sativum* (bulb) Garlic	82.26 (65.06)	84.50 (66.79)	88.54 (70.18)
5.	*Azadirachta indica* (leaf) Neem	15.58 (23.24)	24.43 (29.61)	28.88 (32.49)
	C.D (0.05 level) for Botanicals (B)			0.65
	C.D (0.05 level) for Concentration (C)			1.50
	C.D (0.05 level) for B × C			1.12

Values in parenthesis are arc sine transformed

* Mean of three replications

Table 18: Effect of botanicals on mycelial growth inhibition of *Colletotrichum gloeosporioides*

SI. No	Botanicals	Inhibition of radial growth over control of *C. gloeosporioides* (%)		
		Concentrations (%)		
		10	20	30
1	*Tagetes erecta* (leaf) Mari gold	6.03* (14.21)#	33.46 (35.33)	39.93 (39.17)
2.	*Bougainvillea spectabilis* (leaf) Bougainvillea	5.03 (12.95)	13.29 (21.37)	22.23 (28.12)
3.	*Datura stramonium* (leaf) Datura	37.73 (37.88)	48.79 (44.29)	55.53 (48.15)
		2	4	6
4.	*Allium sativum* (bulb) Garlic	42.19 (40.49)	50.80 (45.44)	62.20 (52.04)
5.	*Azadirachta indica* (leaf) Neem	4.39 (12.10)	8.79 (17.24)	20.09 (26.62)
	C.D (0.05 level) for Botanicals (B)			0.22
	C.D (0.05 level) for Concentration (C)			0.17
	C.D (0.05 level) for B × C			0.39

Values in parenthesis are arc sine transformed

*Mean of three replications

Out of the five extracts tested at one third of their higher doses, maximum per cent inhibition of the fungus 42.19 % was recorded in Garlic at two per cent, followed by Datura at 10 per cent (37.73 %) and they were significantly superior over all other extracts. Other plant extracts performed poorly at their lower doses.

Out of the five extracts tested at two third of their higher doses, highest per cent inhibition 50.80 % was recorded in Garlic at four per cent which was on par with Datura (48.79 %), followed by Mari gold (33.46 %). All other plant extracts were not effective in inhibiting the growth of the fungus.

Out of the five extracts tested at higher doses, maximum per cent inhibition of the fungus 62.20 % was noticed in Garlic, followed by Datura (55.53 %) and Mari gold (39.93 %) (Plate 20). The results revealed that Garlic and Datura were effective at all the concentrations, compared to all other plant extracts tested. It was also observed that with the increase in the concentration of plant extracts used, the effectiveness in the growth inhibition of the fungus was also increasing.

. From the above experiment it is clearly evident that Garlic extract was effective in inhibiting the growth of both the leaf spot pathogens compared to all other plant extracts. It has been selected for compatibility studies and *in vivo* trials.

4.7.3 *In vitro* evaluation of fungicides

Two systemic and five non-systemic fungicides were evaluated at three concentrations viz., recommended dosage, half of the recommended dosage and quarter of the recommended dosage in the laboratory for their efficacy against the pathogens through poisoned food technique.

4.7.3.1 Evaluation of fungicides against *Pestalotiopsis palmarum*

Inhibition of mycelial growth of *P. palmarum* at three concentrations of five systemic fungicides viz., Propiconazole, Azoxystrobin, Difenoconazole, Hexaconazole and Carbendazim and two non-systemic fungicides viz., Copper hydroxide and Mancozeb were used and presented in Table 19; Fig.11. The results revealed that different concentrations of all the fungicides tested, differed significantly in inhibiting the growth of *P. palmarum*

Fig 9: Effect of botanicals on growth inhibition of *Pestalotiopsis palmarum*

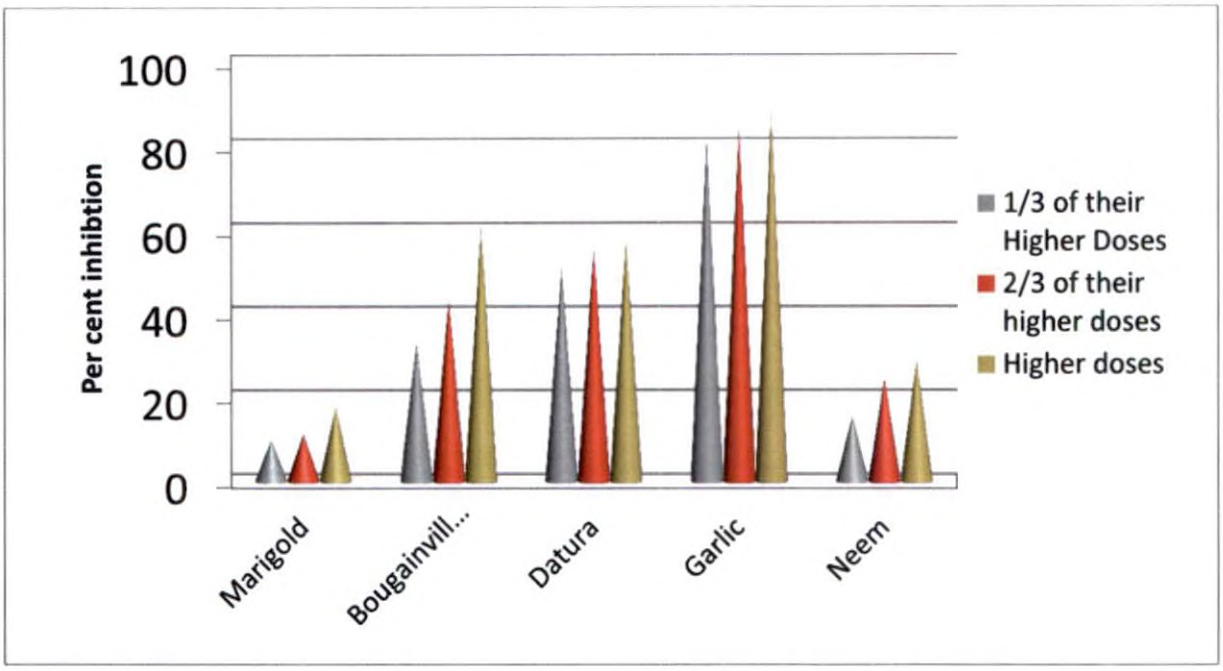


Fig 10: Effect of botanicals on growth inhibition of *Colletotrichum gloeosporioides*

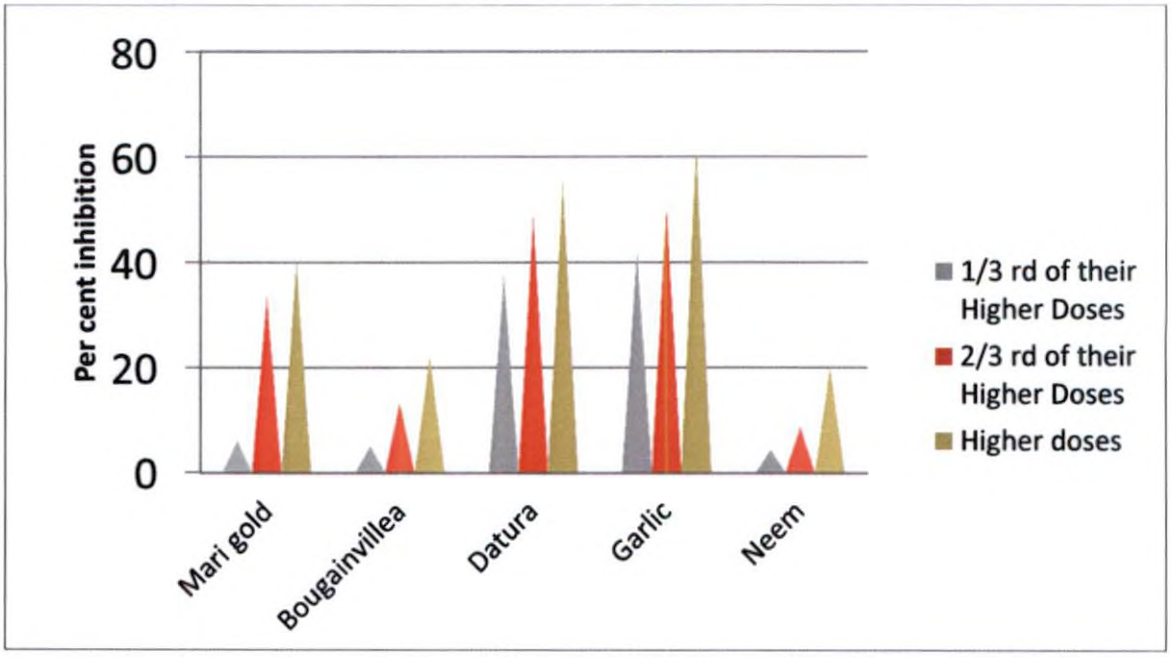


Plate 19: Effect of botanicals on growth inhibition of *Pestalotiopsis palmarum*

Plate 20: Effect of botanicals on growth inhibition of *Colletotrichum gloeosporioides*

At recommended doses of fungicides, complete inhibition of the pathogen was recorded in Propiconazole and Carbendazim which was significantly superior over all other fungicides followed by Difenoconazole (92.30 %), Hexaconazole (90.05 %), Azoxystrobin (78.49 %), Mancozeb (16.65 %) and Copper hydroxide (13.99 %) which were significantly different in their effect.

At half of the recommended dosages Propiconazole and Carbendazim gave complete inhibition on the mycelial growth of the fungus which were significantly superior over other fungicides followed by Difenoconazole (87.84 %).

At quarter of the recommended dosages maximum per cent inhibition of mycelial growth (100 %) of the fungus was recorded in Propiconazole and Carbendazim. Further 0.025 per cent of Azoxystrobin (72.24 %) and Hexaconazole (72.28 %) remain on par with each other. No inhibition was found in Mancozeb @ 0.075 per cent and Copper hydroxide 0.062 per cent. (Plate 21)

At all the tested concentrations of the fungicides Propiconazole and Carbendazim recorded complete inhibition of growth of the fungus, but contact fungicides were not at all effective in any of the tested concentrations in inhibiting the growth of the test fungus.

4.7.3.1 Evaluation of fungicides against *Colletotrichum gloeosporioides*

The results revealed that, there was significant difference in growth inhibition by the different fungicides at their different concentrations on *C. gloeosporioides*. The data are presented in Table 20 and depicted in Fig 12.

At the recommended doses of the fungicides Propiconazole and Carbendazim inhibited cent per cent growth of the fungus. Difenoconazole (88.66 %) and Hexaconazole (86.84 %) remain on par with each other, followed by Copper hydroxide (43.04 %), Azoxystrobin (38.75 %) and Mancozeb (27.51 %).

At half of the recommended dosages, Propiconazole and Carbendazim gave cent per cent inhibition on the fungus, followed by Hexaconazole (82.47 %) and Difenoconazole (80.10 %) remain on par with each other, Copper hydroxide (25.51 %) and Azoxystrobin (24.68 %) remain on par with each other. The least inhibition of fungus was recorded by Mancozeb (10.42 %).

Plate 21: Effect of fungicides on growth inhibition of *Pestalotiopsis palmarum*

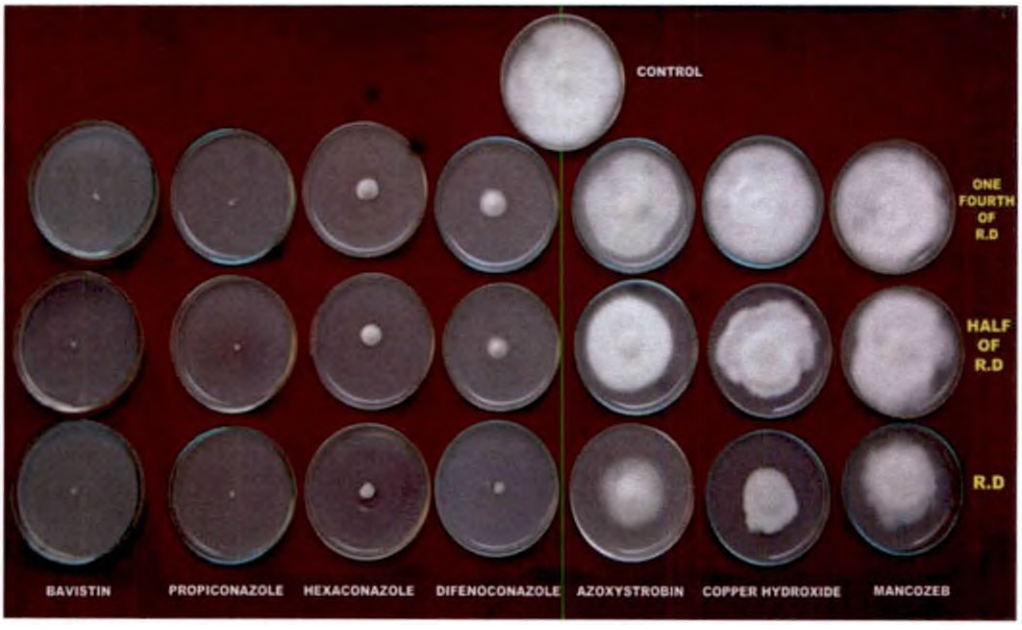


Plate 22: Effect of fungicides on growth inhibition of *Colletotrichum gloeosporioides*

Table 19: Effect of fungicides on mycelial growth inhibition of *Pestalotiopsis palmarum*

S.I. No	Fungicides	Per cent Inhibition of radial growth over control		
		Concentrations (%)		
		Recommended dosage (R D)	Half of the R.D	One fourth of the R.D
1	Copper hydroxide	0.25	0.125	0.062
		13.99# (21.96)*	3.79 (11.23)	0 (0)
2	Mancozeb	0.3	0.15	0.075
		16.65 (24.07)	4.96 (12.87)	0 (0)
3	Propiconazole	0.075	0.037	0.018
		99.99 (90)	99.99 (90)	99.99 (90)
4	Azoxystrobin	0.1	0.05	0.025
		78.49 (62.34)	75.57 (60.35)	72.24 (58.18)
5	Difenoconazole	0.1	0.05	0.025
		92.30 (73.87)	87.84 (69.57)	78.90 (62.23)
6	Hexaconazole	0.1	0.05	0.025
		90.05 (71.59)	82.11 (64.95)	72.28 (58.21)
7	Carbendazim	0.1	0.05	0.025
		99.99 (90)	99.99 (90)	99.99 (90)
	C.D (0.05 level) for Fungicides(F)			1.02
	C.D (0.05 level) for Concentrations (C)			0.67
	C.D (0.05 level) for F × C			1.77

* Values in parenthesis are arc sine transformed

Mean of three replications

R.D – Recommended Dosage

Table 20: Effect of fungicides on mycelial growth inhibition of *Colletotrichum gloeosporioides*

SI.No	Fungicides	Per cent inhibition of radial growth over control		
		Concentrations (%)		
		Recommended dosage(R D)	Half of the R.D	One fourth of the R.D
1	Copper hydroxide	0.25	0.125	0.062
		43.04# (40.98)*	25.51 (30.32)	6.75 (15.05)
2	Mancozeb	0.3	0.15	0.075
		27.51 (31.62)	10.42 (18.82)	7.58 (15.98)
3	Propiconazole	0.075	0.037	0.018
		99.99 (90)	99.99 (90)	99.99 (90)
4	Azoxystrobin	0.1	0.05	0.025
		38.75 (38.48)	24.68 (29.77)	15.65 (23.30)
5	Difenoconazole	0.1	0.05	0.025
		88.66 (70.29)	80.10 (63.48)	76.66 (61.09)
6	Hexaconazole	0.1	0.05	0.025
		86.64 (68.70)	82.47 (65.22)	80.37 (63.37)
7	Carbendazim	0.1	0.05	0.025
		99.99 (90)	99.99 (90)	99.99 (90)
	C.D (0.05 level) for Fungicides(F)			1.06
	C.D (0.05 level) for Concentrations (C)			1.69
	C.D (0.05 level) for F × C			1.83

* Values in parenthesis are arc sine transformed

R.D – Recommended Dosage

Mean of three replications

Fig 11: Effect of fungicides on inhibition of mycelial growth of *Pestalotiopsis palmarum*

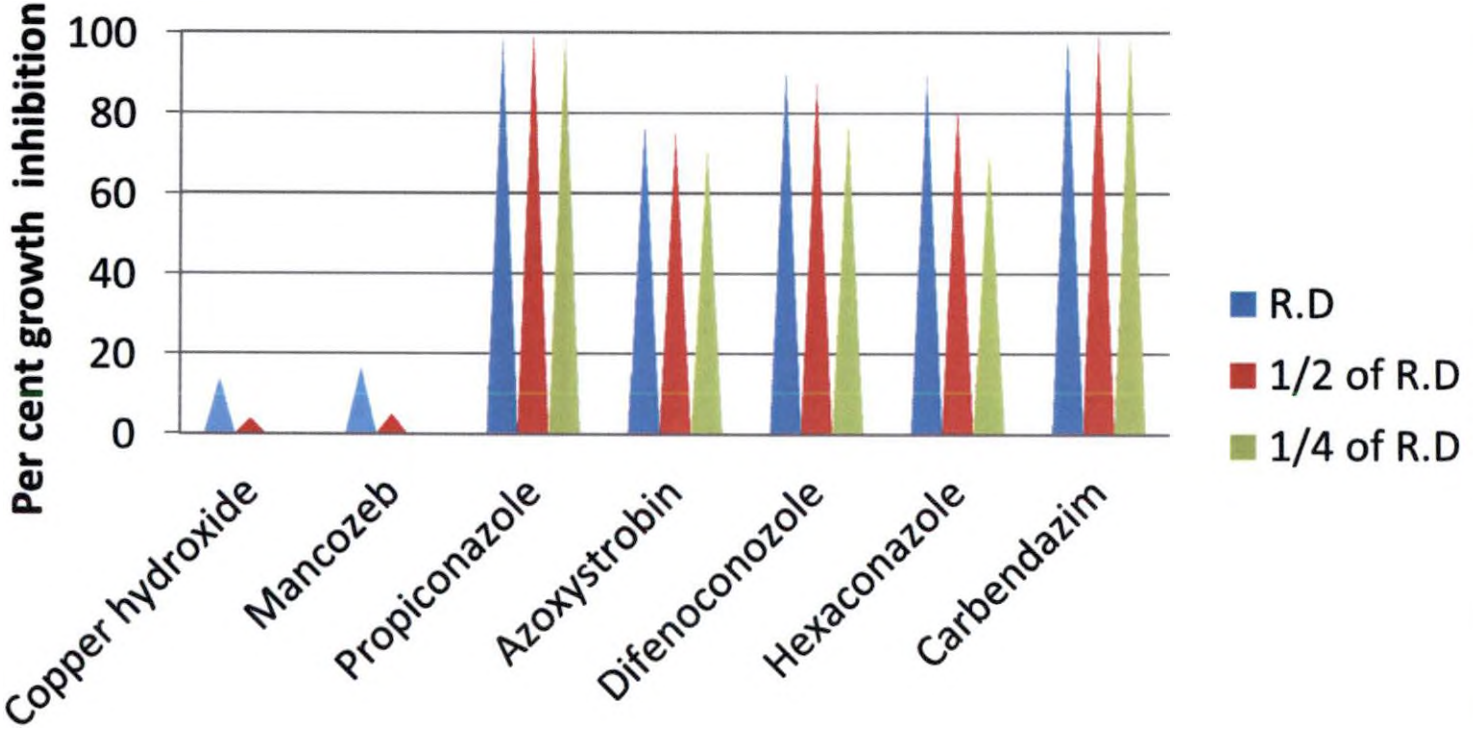
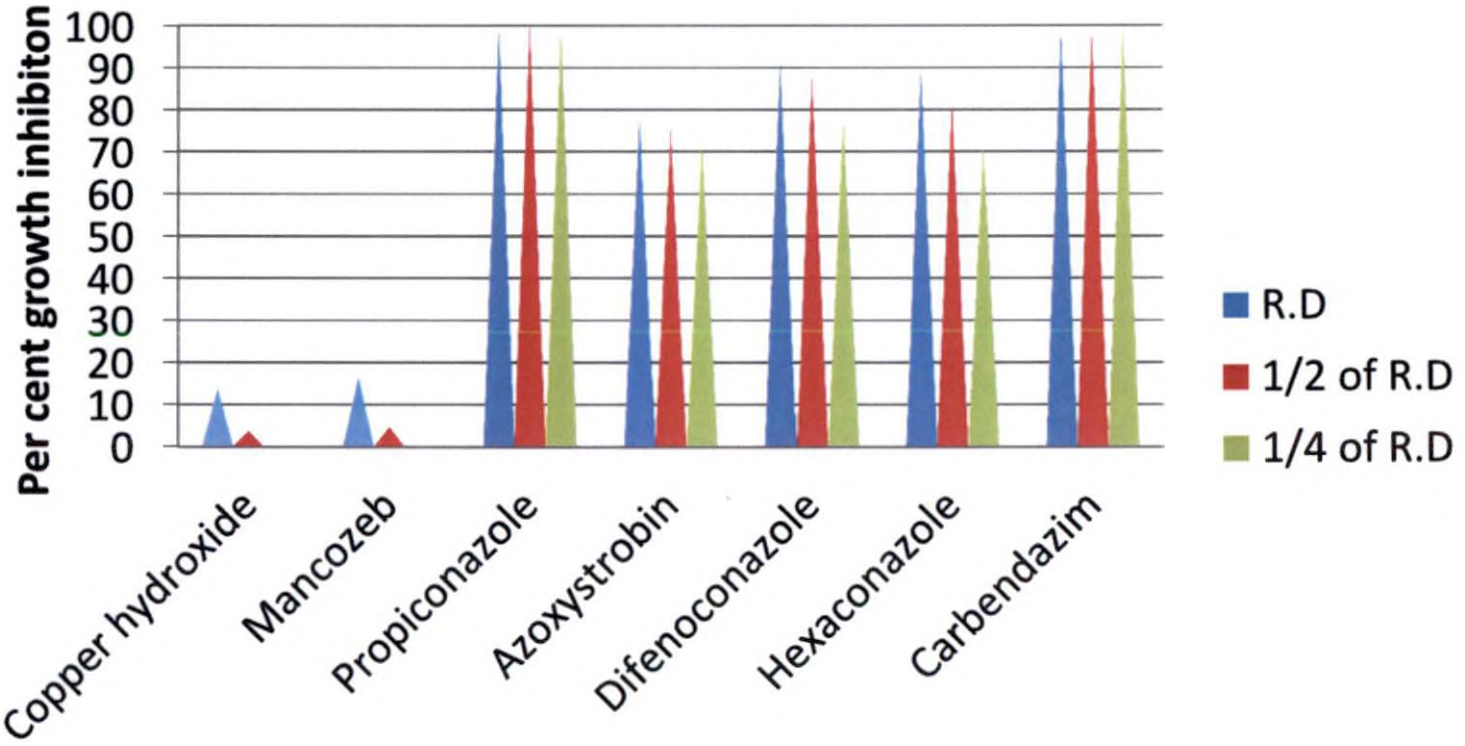


Fig 12: Effect of fungicides on mycelial growth inhibition of *Colletotrichum gloeosporioides*



At quarter of recommended dosages Propiconazole and Carbendazim gave cent per cent inhibition of the fungus followed by Hexaconazole (80.37 %), Difenoconazole (76.66 %). The least inhibition (6.75 %) was found in Copper hydroxide (Plate 22).

At all the tested concentrations, Propiconazole and Carbendazim gave complete inhibition and all other systemic fungicides were effective against both the pathogens except Azoxystrobin but it was found effective against *P.palmarum*. The contact fungicides performed poorly against both the pathogens at all the tested concentrations. So, for compatability studies and *in vivo* evaluation systemic fungicides were selected.

4.7.4 Compatibility of effective fungicides and botanical with *T.harzianum*

In the *in vitro* studies it was observed that among the five botanicals tested, Garlic was effective against both the tested pathogens. Out of the seven chemicals tested, systemic fungicides performed better in inhibiting the growth of the pathogens.

Hence Garlic @ 6 % and systemic chemicals viz., Propiconazole, Azoxystrobin, Difenoconazole, Hexaconazole and Carbendazim at their recommended dosages were selected for compatibility studies with best biocontrol agent *T.harzianum.*

To study the *in vitro* sensitivity of *T.harzianum* to fungicides and botanical poisoned food technique was followed (Shravelle, 1961).

The results of the experiment revealed that (Table 21) (Plate 23), Garlic was least effective in inhibiting the growth of *T.harzianum* (15.79 %), followed by Azoxystrobin (27.90 %) i.e., they were compatible at 82.21 and 72.09 %respectively with the biocontrol agent. All other fungicides completely inhibited the growth of the *T.harzianum* and they are incompatible with the biocontrol agent.

4.8 Management of leaf spot diseases on arecanut seedlings.

Seedlings were inoculated with *P. palmarum* and *C. gloeosporioides* by artificial inoculation on the leaves. Spores were sprayed at 1×10^5 / ml and also mycelial mats were placed on the injured leaves. When the plants start showing the symptoms treatments were applied. Two sprays were given at 15 days interval. The observations on PDI were scored four times, starting from 0^{th} day of spraying, each at 15 days interval and last one 30 days after 2^{nd} spray.

Table 21: Compatibility of effective fungicides and botanical with *Trichoderma harzianum*

SI.No	Chemicals/botanical	Concentration (%)	Inhibition of *T.harzianum* (%)	Compatibility with *T.harzianum* (%)
1	Propiconazole	0.075	100	0
2	Carbendazim	0.1	100	0
3	Hexaconazole	0.1	100	0
4	Difenoconazole	0.1	100	0
5	Azoxystrobin	0.1	27.90	72.09
6	Garlic	6	15.79	82.21
C.D (0.05 level)			1.98	1.98

Mean of three replications

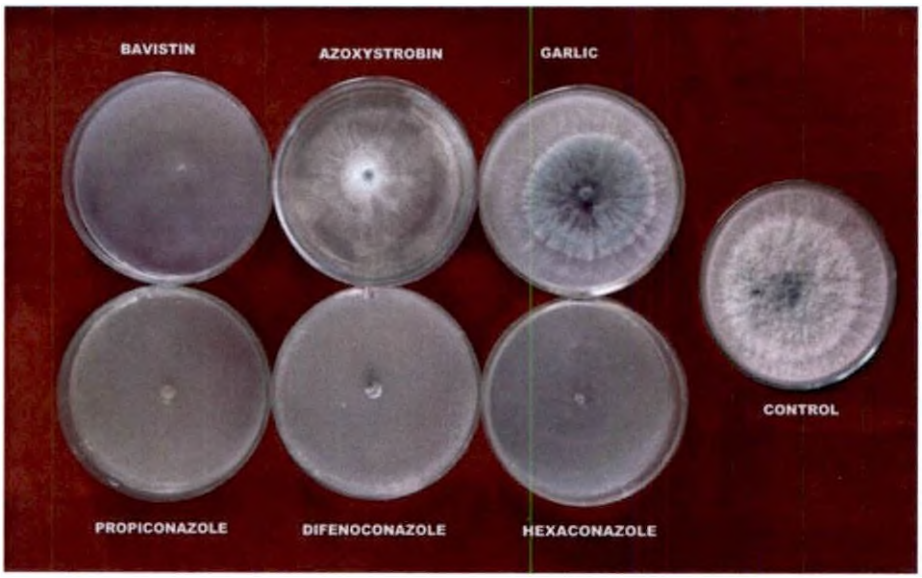


Plate 23: Compatibility of fungicides and botanical with *Trichoderma harzianum*

Plate 24: An overview of management of leaf spot diseases on Areca Seedlings

The results (Table 22) (Fig 13) revealed that 15 days after the first spray treatments T1 (Propiconazole, 0.075 %), T3 (Difenoconazole, 0.1 %), T2 (Carbendazim, 0.1 %) and T4 (Azoxystrobin, 0.1 %) were on par and treatment T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %), T5 (Garlic 6 %) and T8 (Garlic 6 % + *T. harzianum* 2 %) were on par and T8 (Garlic 6 % + *T. harzianum* 2 %) , T6 (*T. harzianum* 2 %) and T9 (control) were on par in their effectiveness in leaf spot control. But treatment T6 (*T. harzianum* 2 %) was significantly inferior over all other treatments in its effectiveness, it was on par with control.

Fifteen days after second spray the disease intensity was decreasing significantly over control in all the treatments. The most effective treatment was T1 (Propiconazole, 0.075 %), followed by T2 (Carbendazim, 0.1 %), T3 (Difenoconazole, 0.1 %), and T4 (Azoxystrobin, 0.1 %).

Thirty days after third spray the observations on disease intensity showed that all the treatments were effective in controlling the leaf spot diseases. T2 (Carbendazim, 0.1 %) recorded minimum disease intensity 15.13 per cent, followed by T1 (Propiconazole, 0. 075 %) 15.13 per cent and T3 (Difenoconazole, 0.1 %) 15.92 per cent, they were on par with each other. These treatments were significantly superior over all other treatments.

The most effective treatment was T2 (Carbendazim, 0.2 %) which had 50.15 per cent reduction in PDI and this was on par with T1 (Propiconazole, 0.075 %) which recorded 48.61 per cent reduction in PDI and T3 (Difenoconazole, 0.1 %) with 48.29 per cent , followed by T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %)which recorded 41.50 per cent reduction in PDI over control and pretreatment. T6 (*T. harzianum* 2 %) performed poorly compared to all other treatments in controlling the leaf spot diseases with only 22.07 per cent reduction in PDI (Plate 24).

4.9 Management of leaf spot diseases on young palms in the field

A preliminary survey was undertaken in the I.F. Vellayani, to score the PDI of the palms. The arecanut palms having equal intensity of the disease were selected for the experiment. Three spray of treatments was done and observations was taken at five intervals.

An observation on PDI was taken before spray, after the sprays and last one 30 days after 3rd spray. Sticker solution was added into the spray solution at 1 ml/L, to make spray solution stick on to the plant (Plate 25).

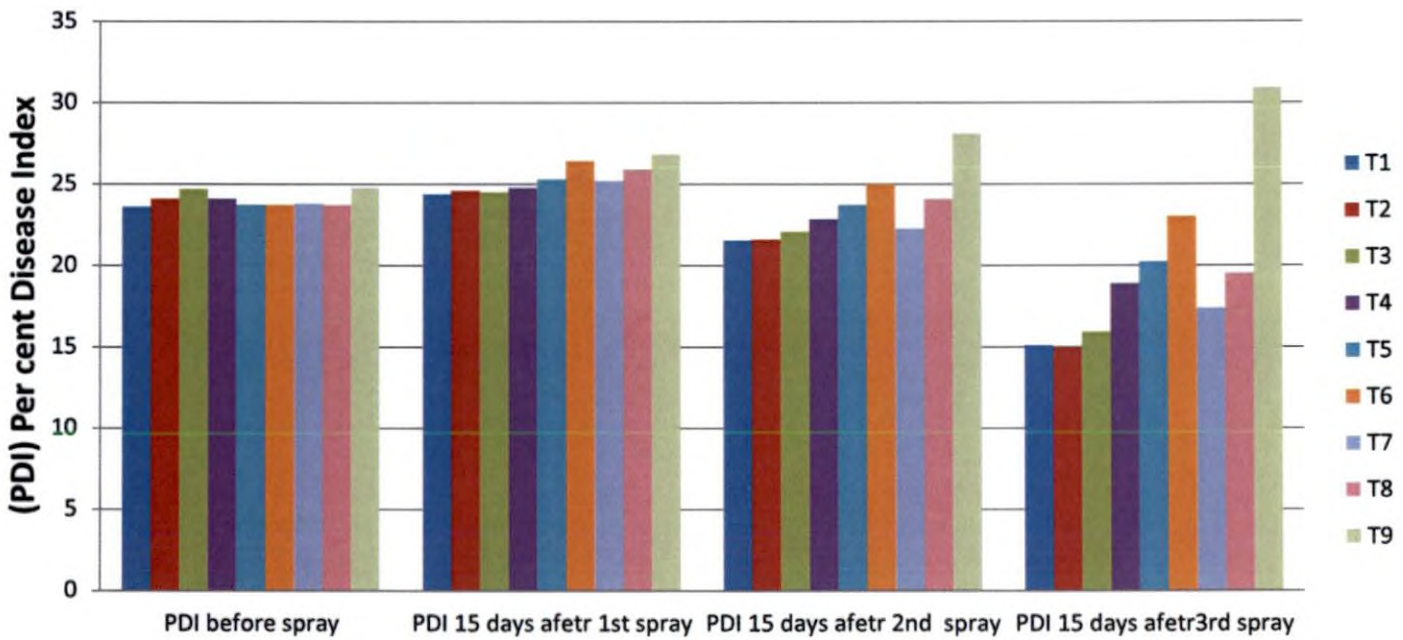
Table 22: Effect of fungicides, bioagents and their combinations on leaf spot diseases of arecanut seedlings.

SI.No	Treatment	PDI# before spray	PDI 15 days after 1st spray	PDI 15 days after 2nd spray	PDI 30 days after 2nd spray	Per cent reduction over control and pre treatment
1	T1- Propiconazole (0.075%)	23.62 (4.86)*	24.40 (4.94)	21.53 (4.64)	15.13 (3.89)	48.61
2	T2- Carbendazim (0.1%)	24.11 (4.91)	24.60 (4.96)	21.62 (4.65)	14.98 (3.87)	50.15
3	T3- Difenoconazole (0.1%)	24.70 (4.97)	24.50 (4.95)	22.09 (4.70)	15.92 (3.99)	48.29
4	T4- Azoxystrobin (0.1%)	24.11 (4.91)	24.80 (4.98)	22.85 (4.78)	18.92 (4.35)	37.04
5	T5- Garlic (0.1%)	23.72 (4.87)	25.30 (5.03)	23.72 (4.87)	20.25 (4.50)	31.50
6	T6- *T. harzianum* (2%)	23.72 (4.87)	26.42 (5.14)	25.0 (5.00)	23.04 (4.80)	22.07
7	T7-Azoxystrobin (0.1%) + *T. harzianum* (2%)	23.81 (4.88)	25.20 (5.02)x	22.28 (4.72)	17.39 (4.17)	41.40
8	T8- Garlic (6%) + *T. harzianum* (2%)	23.72 (4.87)	25.91 (5.09)	24.11 (4.91)	19.54 (4.42)	33.91
9	T9- control	24.80 (4.98)	26.83 (5.18)	28.11 (5.33)	30.91 (5.56)	
C.D at 0.05 level			0.095	0.14	0.32	

PDI = Per cent Disease Index

* Values in parenthesis are square root (√) transformed values

Fig 13: Effect of fungicides, bioagents and their combinations on leaf spot diseases of arecanut seedlings.



T1- Propiconazole
T2- Carbendazim
T3- Difenoconazole
T4- Azoxystrobin
T5- Garlic

T6- *T. harzianum*
T7-Azoxystrobin (0.1%) +*T. harzianum* (2%)
T8- Garlic (6%) + *T. harzianum* (2%)
T9- control

Fifteen days after first spray, the results (Table 23) (Fig 14) revealed that treatment T2 (Carbendazim, 0.2 %), T1 (Propiconazole, 0.075 %), T3 (Difenoconazole, 0.1 %) and T4 (Azoxystrobin, 0.1 %) were highly effective compared to T5 (Garlic 6 %), T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %) and T8 (Garlic 6 % + *T. harzianum* 2 %) and they were on par with each other also. The effect of treatment T5 (Garlic 6 %), T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %) and T8 (Garlic 6 % + *T. harzianum* 2 %) were on par with control.

After 2nd spray the results revealed that all the treatments were highly effective compared to control. The most effective treatment was T2 (Carbendazim, 0.2 %) which was on par with T1 (Propiconazole, 0.075 %) followed by treatment T3 (Difenoconazole, 0.1 %), T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %) and T4 (Azoxystrobin, 0.1 %) which were on par and all these treatments were highly effective than T8 (Garlic 6 % + *T. harzianum* 2 %), T6 (*T. harzianum* 2 %) and T5 (Garlic 6 %) which were on par with each other.

3rd spray was given 15 days after the 2nd spray and the results revealed that all treatments were highly effective in controlling leaf spot disease of arecanut when compared with control. The most effective treatment was T2 (Carbendazim, 0.2 %) which was on par with T1 (Propiconazole, 0.075 %) and these two were significantly superior over all the other treatment. These were followed by treatment T3 (Difenoconazole, 0.1 %) and T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %) which were on par with each other followed by T4 (Azoxystrobin, 0.1 %). But T3 (Difenoconazole, 0.1 %), was significantly superior over T4 (Azoxystrobin, 0.1 %) in its effect. T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %) and T4 (Azoxystrobin, 0.1 %) were on par with each other. Then these were followed by T8 (Garlic 6 % + *T. harzianum* 2 %), T5 (Garlic 6 %) and T6 (*T. harzianum* 2 %) in descending order and they were on par with each other. But the effect of treatment T4 (Azoxystrobin, 0.1 %) was significantly superior over T6 (Garlic 6 %) the least effective treatment.

Thirty days after 3rd spray was given and the results revealed that trend of effectiveness of treatments observed after 15 days were maintained in controlling the leaf spot diseases of arecanut. All the treatments were significantly effective when compared to control. T2 (Carbendazim, 0.2 %) recorded minimum disease intensity 25.42 per cent, followed by T1 (Propiconazole, 0.075 %) 25.86 per cent) and they were on par with each other, followed by T7 (Azoxystrobin 0.1 % + *T. harzianum* 2 %) 21.25 per cent.

Plate 25 a.. Field view of young areca palms (Management trial)

Plate 25 b.: Field view of young areca palms (Management trial)

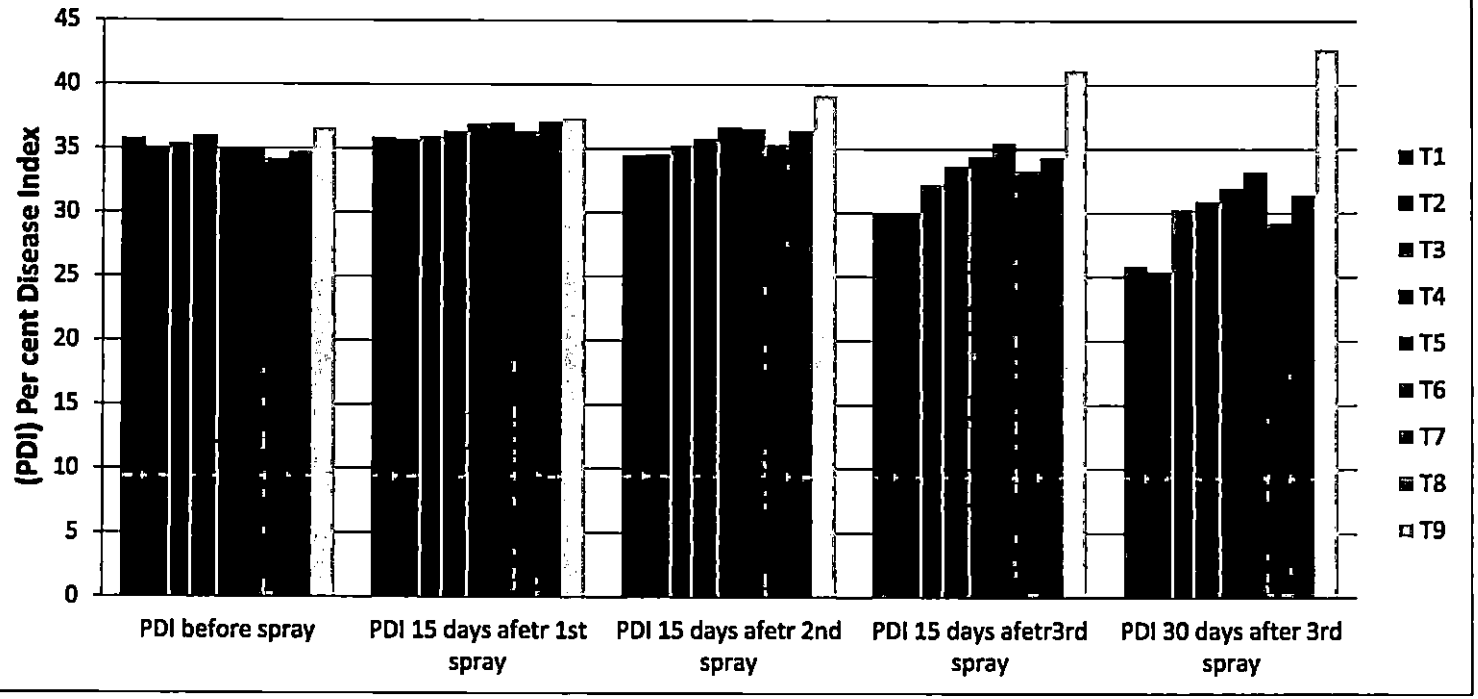
Table 23: Effect of fungicides, bioagents and their combinations on the leaf spot diseases of young areca palms

SI.No	Treatment	#PDI before spray	PDI 15 days after 1st spray	PDI 15 days after 2nd spray	PDI 15 days after 3rd spray	PDI 30 days after 3rd spray	Per cent reduction over control and pre treatment
1	T1- Propiconazole (0.075%)	35.76 (5.98)	35.87 (5.98)*	34.49 (5.87)	30.07 (5.48)	25.86 (5.08)	38.90
2	T2- Carbendazim (0.1%)	35.05 (5.92)	35.67 (5.97)	34.56 (5.87)	29.93 (5.47)	25.42 (5.04)	39.33
3	T3- Difenoconazole (0.1%)	35.40 (5.95)	35.94 (5.99)	35.30 (5.94)	32.21 (5.67)	30.30 (5.50)	28.05
4	T4- Azoxystrobin (0.1%)	36.0 (6.00)	36.35 (6.02)	35.76 (5.98)	33.69 (5.80)	30.92 (5.56)	27.19
5	T5- Garlic (0.1%)	34.93 (5.91)	36.92 (6.07)	36.72 (6.05)	34.42 (5.86)	31.96 (5.65)	23.59
6	T6- *T. harzianum* (2%)	34.93 (5.91)	37.05 (6.08)	36.57 (6.04)	35.50 (5.95)	33.25 (5.76)	20.51
7	T7-Azoxystrobin (0.1%) + *T. harzianum* (2%)	34.22 (5.85)	36.34 (6.02)	35.35 (5.94)	33.33 (5.77)	29.25 (5.40)	29.36
8	T8- Garlic (6%) + *T. harzianum* (2%)	34.81 (5.90)	37.11 (6.09)	36.47 (6.03)	34.38 (5.86)	31.47 (5.61)	24.64
9	T9- control	36.61 (6.05)	37.35 (6.11)	39.17 (6.25)	41.18 (6.41)	42.82 (6.54)	
C.D at 0.05 level			0.058	0.094	0.11	0.17	

* Values in parenthesis are square root (√) transformed values.

PDI = Per cent Disease Index

Fig 14: Effect of fungicides, bioagents and their combinations on the leaf spot diseases of young areca palms



T1- Propiconazole
T2- Carbendazim
T3- Difenoconazole
T4- Azoxystrobin
T5- Garlic
T6- *T. harzianum*
T7-Azoxystrobin (0.1%) +*T. harzianum* (2%)
T8- Garlic (6%) + *T. harzianum* (2%)
T9- control

The most effective treatment was T2 (Carbendazim, 0.2 %) which had 39.33 per cent reduction in PDI, which was on par with T1 (Propiconazole, 0.075 %) which recorded 38.90 per cent reduction in PDI, followed by T7 (Azoxystrobin,0.1 %+*T.harzianum* 0.2 %) which had 29.36 per cent reduction in PDI over control and pre- treatment. T6 (*T.harzianum* 0.2 %) performed poorly compared to all other treatments in controlling the leaf spot diseases with only 20.51 per cent reduction in PDI. These treatments were superior over T7 (Azoxystrobin, 0.1 % + *T.harzianum* 0.2 %), T3 (Difenoconazole, 0.1 %) and T4 (Azoxystrobin, 0.1 %) which were on par with each other followed by T8 (Garlic 6 % + *T. harzianum* 2 %), T5 (Garlic 6 %) and T6 (*T. harzianum* 2 %). The effect of T7 (Azoxystrobin, 0.1 %+*T.harzianum* 0.2 %), T3 (Difenoconazole, 0.1 %) and T4 (Azoxystrobin, 0.1 %) were significantly superior over T8 (Garlic 6 % + *T. harzianum* 2 %), and the effect of T4 (Azoxystrobin, 0.1 %), T8 (Garlic 6 % + *T. harzianum* 2 %) and T5 (Garlic 6 %) were significantly superior over T6 which was the least effective treatment.

Discussion

5. DISCUSSION

Arecanut (*Areca catechu* L.) is a perennial crop cultivated in India and has considerable economic importance. The crop suffers from many foliar diseases caused by various phytopathogenic fungi leading to poor crop stand and yield loss. The present investigation on "Management of leaf spot diseases of Arecanut" was undertaken with the following objectives.

1. To study the leaf spot diseases affecting young areca palms and

2. To develop an integrated management practice to contain the diseases.

The arecanut leaf samples showing leaf spot symptoms were collected from different arecanut gardens located in Instructional Farm at Vellayani, Peringamala in Thiruvananthapuram and Balussery in Calicut district.

The fungi *Pestalotiopsis palmarum*, *Colletotrichum gloeosporioides* were obtained from all the surveyed locations and *Phomopsis palmicola* was obtained from the Balussery area in Calicut district and the cultures were maintained in PDA medium.

The leaf spots of *Pestalotiopsis palmarum* were round to oval, brown to dark brown with dark brown margin. The spots were characterized with grey coloured centre and with numerous fruiting bodies. These spots were noticed only on the leaves of outer whorl. Similar symptoms by *Pestalotia palmarum* on arecanut leaf were reported by Ashoka, 2003 and Islam et al., 2004 and on coconut leaf by Obazze and Ikozun, 1985 and Praveena, 1999. Menon et al. (1962) reported the withering of affected tissues, production of pustules of the fungus and shredding of entire lamina in arecanut.

The symptoms due to *Colletotrichum gloeosporioides* included small circular or oblong to irregular brownish spots. The centre of the spots turned light grey or straw colour without any outer ring, some plants were showing brown or grey spots surrounded by an yellow halo. In the advanced stages spots coalesced to give a blighted appearance from tip downwards. Similar type of symptoms were recorded on arecanut by Ashoka (2003), Hegde and Hegde (1986).

Rectangular to irregular, light to dark brown lesions on the leaf lamina, midrib and basal fronds followed by increased severity was reported by Hegde et al. (1988). However, presence of yellow halos was reported by Padalkar et al. (1996).

In the present study, the symptoms of *Phomopsis palmicola* on arecanut includes more or less round to oval spots with brownish margin and light brown or straw coloured centre. These spots were confined to leaf tip and on coalescence extended up to 15 cm from the tip. This was followed by withering and shredding of affected leaves leading to premature death of the entire leaf let. Similar reports were made by Ashoka, (2003). Roy (1965) from Assam described that *P. palmicola* produced brown, more or less round spots of variable size and a few spots coalesced to form bigger irregular greyish blights with dull black minute pycnidia. Hussain et al. (1992) reported the leaf blight of arecanut caused by *P. palmicola* in Bangladesh.

In the present study, to prove the pathogenicity of all the three pathogens artificial inoculation were done on healthy arecanut seedlings with prior injury. The symptoms produced by *P. palmarum* were minute brown spots four days after the inoculation on the wounded leaves. After eight days the spots were enlarged with ashy brown centre surrounded by brown margin. Ashoka (2003) and Chowdhury (1946) conducted experiments with *P. palmarum* on *Areca catèchu* and found that inoculation was successful only through injury indicating the fungus is wound penetrant. Similar reports were made by Praveena (1999) and Obazze and Ikozun (1985) for *Pestalotiopsis palmarum* on coconut leaves.

In pathogenicity test, *C. gloeosporioides* produced the spots, which were small, irregular and brownish surrounded by yellow halo on the ninth day of inoculation. This result is in line with the works of Ashoka (2003), Padalkar et al. (1996) and Ramanujam and Chandramohan (1987).

Phomopsis palmicola produced typical minute brown spots seven days after the inoculation on the leaves with injury, also exhibited the requirement of wound to cause infection. Similar results were reported by Ashoka (2003) and Roy (1965).

On PDA, the mycelial growth of *P. palmarum* was greyish to white cottony zonate mycelium with the production of black coloured acervuli after four to five days of inoculation. Conidia were five celled with three coloured intermediate cells. Average measurement of conidia was 23.5 µm × 6.5 µm, the upper end of the conidia bears two to three long, slender, colourless appendages and they were 20 µm in length. Similar results were obtained by Ashoka (2003), Islam (2004), Carl and Bartlett (1922), Chowdury (1946) and Praveena (1999) found that all the characters of the fungus were similar with the earlier reports.

On PDA, the growth of *C gloeosporioides* was dense, cottony, dirty white to greyish aerial mycelium with even margin and production of pinkish droplets of acervuli was also seen. The conidia were oblong or cylindrical or slightly dumbel, hyaline, aseptate with rounded ends and with one or two oil globules and it measured 15.74 µm × 5.43 µm. These characters were similar with the earlier descriptions by Hegde and Hegde (1986), Hegde et al. (1988) and Pradeepkumar (2000), Sunil kulkarni (2009), Jayalaxmi (2009).

On PDA, the growth of *P. palmicola* was white, thin to moderate mycelium with flat mycelial mats throughout the culture plate with even margins. The conidia were hyaline, single celled, uninucleate and measuring 7.20 µm × 2.20 µm. These characters were similar with the descriptions of Ashoka (2003) and Roy (1965) on arecanut.

In the present study sequencing of ITS region of three leaf spot pathogens isolates namely *Pestalotiopsis palmarum, Colletotrichum gloeosporioides and Phomopsis palmicola* were done to molecularly characterize them and their identity was molecularly confirmed in NCBI database and sequences have been deposited in the Gen Bank database and the accession numbers have been provided to the isolates by NCBI. The results from the Phylogenetic tree revealed that isolate 1 (*Pestalotiopsis palmarum* from Vellayani) was a totally different organism and that has been shown to be very different in sequence to the other organisms. Isolates CC1 and CC2 (*Colletotrichum gloeosporioides*) found from Balussery, Calicut were showing some similarity in their sequences and they had

less similarity with VC (*C. gloeosporioides*, from Vellayani). Isolates CP1, and CP2 (*Phomopsis palmicola* from Calicut) had higher similarity in their sequences when compared with PV (*P. palmicola* from Vellayani). Earlier studies made by Meena et al. (2012) *Trichoderma* isolates collected from different regions showed variations in their nucleotide sequences, Sarvottam et al. (2009) observed the genetic diversity among the isolates of *Pestalotiopsis* spp. collected from tea growing areas, Rohana et al. (2005) reported the variations in *Colletotrichum* sp collected from different locations, Li et al. (2001) reported the genetic diversity in the isolates obtained from different geographical regions of *Endocronartium harkneii*, causal agent western gall rust fungus, these results shown that organisms in one locality differed from another locality. In the same way CC1 and CC2 (*Colleotrichum gloeosporioides*, Balussery Calicut) found from same locality showed sequence similarity while VC (*C. gloeosporioides* from Vellayani) showed less similarity. Isolates CP1 and CP2 found in same locality (*Phomopsis palmicola,* Balussery Calicut) found in same geographical region showed higher similarity, than with PV (*P . palmicola* , Vellayani). More genetic diversity was noticed among the isolates obtained from different regions.

Fungi secure food and energy from the substrate upon which they live in nature. In order to culture the fungus in the laboratory it is necessary to furnish those essential elements and compounds in the medium for their growth and other life processes. Neither all media are equally good for all fungi, nor there is a universal substrate or artificial medium upon which all fungi can grow. So, 10 different media were used for growth of *C. gloeosporioides.*

Among the various media used for growth of *C. gloeosporioides,* PDA, Czapek's agar, Richard's agar, Sabouraud's agar and Corn meal agar were proved to be best for good growth. Present studies are in accordance to the better performance of *C. gloeosporioides* on PDA as reported by Jayalakshmi (2010), Ekbote et al. (1997). Any living organism requires a particular medium with optimum pH for growth and development. Good growth of *P. palmarum* was observed at acidic to basic pH levels (i.e., 5.0 to 6.5). There was decline in the

growth of the fungus towards the alkaline pH. This rate of decrease of mycelial growth in alkali pH levels indicates that this fungus liked mild acid for its growth. These results were similar with those recorded by Sarkar (1960), Mhaskar and Rao (1981), Mishra and Chhotary (1989)) and Sridhara gupta (2000).

A wide range of pH supported the growth of *C. gloeosporioides*, good growth was found at 5.0 to 7.0 pH. The optimum pH range was obtained towards acidic pH side and sudden decline were observed towards basic pH side which indicated that fungus was acid tolerant. Cochrane (1958) and Bilgrami and Verma (1978) also opined that in contrast to bacteria and actinomycetes, fungi are relatively more tolerant to acid ions (H^+) than basic ions (OH^-). The observations are in agreement with those of Singh and Shukla (1986), Ekbote (1994) and Sunil kulkarni (2009).

The leaf spot diseases on arecanut were observed on palms below 10 years age and the intensity varied from place to place. The disease incidence recorded ranged from 75 to 85 per cent. This is in line with the work of Papa Rao and Govinda Rao (1966) in Andrapradesh, Ashoka (2003) in Karnataka. In the present investigation maximum infection of leaf spot diseases was seen on the plants aged below 10 years and the lower most leaves had the maximum infection (38.51 PDI and 27.68 PDI on 8^{th} and 7^{th} leaf respectively) while the top leaves were either remained free or were infected occasionally. These findings did not differ from that of Bhat (1983 and 1988), Bhat et al. (1992), Hebbar (1992) and Ashoka (2003). This may be due to depletion of sugars in the older leaves.

Survey conducted in the Instructional Farm, Vellayani revealed that the disease occurred all-round the year and PDI ranged from 20.13 to 33.21 in the different months of the year 2012-13. It was observed that disease gradually increased from June to November from 21.90 to 33.21 %. During this period many fresh leaf spots were formed. After November the disease was gradually decreasing up to May. Formation of fresh infections declined during the same period.

The maximum disease intensity of 33.21 was observed in November when weather recorded maximum temperature (30.2°C), minimum temperature (23°C), Relative Humidity (96.4%) and Rainfall (111.1 mm). Lowest intensity was recorded during May, when the weather data showed maximum temperature (32.1°C) and minimum temperature (25.7°C), Relative Humidity (91 %) and Rainfall (49 mm). Rao et al. (1976) recorded highest number of infections of *P. palmarum* occurred in August, moderate during September and October, again became severe during November. But during December there were very few fresh leaf spots and none at all from January. They presumed that the disease manifests mostly during the cool and humid months and extensive blights during summer is only a secondary effect.

Correlation was worked out with weather data and PDI. The results are presented in the Table 13. It was observed that maximum temperature and minimum temperature were negatively correlated and relative humidity and Rainfall were positively correlated with PDI.

No earlier research work is there in Kerala on arecanut to correlate it with weather data. However, a comparison was made with the similar work done on coconut. According to Praveena (1996) grey leaf spot disease of coconut gradually increased from June to January and in subsequent months blighting of leaves was observed and negative correlation was observed between weather parameters and PDI. Similarly Suryachandraselvan et al.(1991) reported that intensity of grey leaf spot on coconut was maximum in December (40.5 %) and minimum in June (23.9 %)). They had mentioned a highly significant negative correlation between disease intensity, relative humidity and rainfall. Thakur (1988) reported that the intensity of the mungbean anthracnose has been found negatively correlated with the temperature.

Use of bioagents, now a days, is best and has been most emphasized and widely accepted practice as it is environmentally safe and can overcome the residual problems associated with the heavy use of fungicides for management of

the diseases. Hence, the present investigation was taken up to screen the bioagents for effective management of leaf spot diseases of arecanut.

Among the bioagents tested against *Pestalotiopsis palmarum*, *Trichoderma harzianum* was found to be the best in inhibiting the mycelial growth (62.77%). This observation is in accordance with the earlier reports by Meena (2009), Saju et al. (2011) on the ability of *Trichoderma* sp. in inhibiting *Pestalotiopsis* sp.

Among the different bioagents assessed against *C. gloeosporioides*, *T. harzianum* has inhibited the growth of fungus with maximum extent (51.04%) followed by Bacteria (B1) (46.72%). The present investigation are in agreement with Gud and Raut (2008), who found effectiveness of *T. harzianum* against *C. gloeosporioides*, whereas Laxman (2006) found it effective against *C. truncatum* and Gupta (1991) reported it against *C. lindemuthianum*.

At present, plant extracts are gaining importance in plant disease management practices. These are cheaper and safer means of disease management which reduce not only toxicity hazards but also present eco-friendly approach in nature.

In the present investigation though complete inhibition of *Pestalotiopsis palmarum* was not observed in any of the five botanicals used, considerable amount of inhibition was noticed in some of the botanicals. Garlic (bulb) extract at 2, 4 and 6 % had maximum growth inhibition of 82.26 %, 84.50 %, and 88.54 % respectively on *P. palmarum*, leaf extracts of Neem and Marigold performed poorly against this pathogen at all the three concentrations tested. These results are in accordance with Islam (2004) who reported that Garlic at 5 % gave complete inhibition on the growth of *Pestalotia palmarum* the leaf spot pathogen of betelnut and he also reported that Neem leaf extract at 5% was not effective.

Out of five botanicals tested at three different concentrations against *C. gloeosporioides* maximum inhibition was observed in Garlic bulb extract at 6% with 62.20 per cent followed by leaf extracts of Datura at 30 % with inhibition of

55.53 per cent. These results are in accordance with earlier reports by Prashanth et al (2008) who observed the effectiveness of Garlic extract and Datura leaf extract on inhibiting the growth of *C. gloeosporioides*. Further, Jadav et al. (2008) also opined that ability of Garlic bulb extract (10%) in inhibiting the growth of *C. gloeosporioides,* and Gupta et al. (1981) recorded that Garlic was effective in inhibiting *C. capsici*

The effectiveness of garlic as a pesticide due to an acrid volatile oil which contains diallyl disulphide, diallyl trisulphide and sulphoxides derived from allicin has been well established. (Venkataravanappa, 2002).

In vitro evaluation of fungicides provides useful and preliminary information regarding efficacy of fungicides against pathogen within a shortest period of time and therefore, serves as a guide for field testing. In the present investigation , five systemic and two non-systemic fungicides were tested at three concentrations on *Pestalotiopsis palmarum* and *C. gloeosporioides.*

Among the seven fungicides tested, Propiconazole at (0.075%, 0.037%, 0.018%) and Carbendazim at (0.1%, 0.05%, 0.025%) completely inhibited the growth of *P. palmarum*. These results are in agreement with earlier made by Khalequzzaman et al. (1998), Praveena (1999), Ashoka (2003), Islam et al (2004). Further, the efficacy of Carbendazim was reported by Das and Mahanta (1985), Kudalkar et al., (1991), Anupama (1997) and Karthikeyan and Bhaskaran (1998) on *P. palmarum*, Saju et al. (2011) reported that Carbendazim at 0.1% completely inhibited the growth of *Pestalotiopsis* sp. causing leaf streak disease in cardamom. Mancozeb and Copper hydroxide performed poorly in inhibiting the growth of pathogen. The ineffectiveness of Mancozeb in inhibiting the growth of *P. palmarum* was recorded by Suryachandraselvan et al. (1993) and Islam et al. (2004).

Among seven fungicides tested against *Colletotrichum gloeosporioides* Propiconazole at (0.075%, 0.037%, 0.018%) and Carbendazim at (0.1%, 0.05%, 0.025%) were the best in inhibiting (100%) the growth. These results were

supported by the earlier reports made by Goswami et al. (1996), Pradeep kumar (2000), Ekbote et al. (1996), Gud and Raut (2008), Patel (2009) and Watve et al. (2009), Sunil kulkarni (2009), that Carbendazim and Propiconazole completely inhibited the growth of *C. gloeosporioides*. Contact fungicides Mancozeb and Copper hydroxide performed poorly in inhibiting the growth of *C. gloeosporioides*. The findings were in conformity with that of Saraswathi and Radhakrishnan Nair (1973) reported that Dithane M- 45 at 2000 ppm was not effective against *Colletotrichum gloeosporioides*, inflorescence die- back pathogen in arecanut.

The effectiveness of the Triazole fungicides like propiconazole may be attributed to their interference with the biosynthesis of fungal sterols and inhibit the ergosterol biosynthesis. In many fungi, ergosterol is essential for the structure of cell wall and its absence cause irrepairable damage to cell wall leading to death of fungal cell. A similar study was reported for the effectiveness of Triazoles, which inhibit the sterol biosynthesis pathway in fungi (Nene and Thapliyal, 1973).

The effectiveness of Carbendazim in growth inhibition on the pathogens is characterized by the inhibition of spindle formation during mitosis and thereby killing the pathogen (Kalim et al., 2000).

In the compatibility studies, systemic fungicides like Propiconazole (0.075%), Carbendazim (0.1%), Difenoconazole and Hexaconazole (0.1%) completely inhibited the growth of *T. harzianum*. These results are similar to the earlier reports made by Sarkar et al.(2010) and Bheemaraya et al. (2012). Whereas Azoxystrobin (0.1%) and Garlic (6%) were compatible at 72.09 per cent and 83.20 per cent with *T. harzianum*. These results are in agreement with report by Ranganathswamy et al. (2012) that Azoxystrobin (0.1%) inhibited only 37.7 % growth of *T. harzianum*. Archana et al. (2012) observed that *T. viride* is completely compatible with Azoxystrobin 23SC upto 15 ppm. Bheemaraya (2012) reported that NSKE, Pongamia leaf extract and Eucaluptus leaf extract were compatible with *Trichoderma* sp. Relatively high sensitivity of *T. harzianum* may

be due to the fact that the isolate was obtained from Arecanut gardens which were less exposed to the fungicidal application.

The fungicides, botanical, bioagent which were found effective in laboratory condition and with their compatible combinations were evaluated in the greenhouse conditions for the management of leaf spot diseases of arecanut in the seedling level. The results, thirty days after the second spray revealed that foliar spray of Carbendazim (0.1%) on leaf spot affected seedlings significantly reduced the disease incidence, whereas Propiconazole (0.075%) and Difenoconazole (0.1%) remained statistically on par with each other, followed by combination of *T. harzianum* (2%) + Azoxystrobin (0.1%).

Four fungicides, one botanical, one bioagent and two combination treatments were evaluated against the leaf spot diseases of arecanut in the field level. Results of the experiment revealed that on thirty days after third spray, plants sprayed with Carbendazim (0.1%) and with Propiconazole (0.075%) had significant reduction in the disease intensity compared to all other treatments, followed by combination of *T. harzianum* (2%) + Azoxystrobin (0.1%) and Difenoconazole (0.1%). These results are in agreement with the reports made by previous workers Anxianshu and Hanlianjian (1994) whereas spraying of Carbendazim, Bordeaux mixture and Chlorothalonil managed the Pestalotia leaf spot in coconut, Khalequzzaman et al. (1998) reported that Carbendazim (0.1%) was effective in controlling *P. palmarum* leaf spot pathogen in coconut, Sanjay et al. (2008) reported that Carbendazim (0.05%) effectively managed grey blight disease in tea. Goswami et al. (1996) revealed that Propiconazole (0.2%) reduced the leaf spot in arecanut caused by *Colletotrichum arecae,* Prashanth et al. (2008) reported that Difenoconazole and Propiconazole was effective in controlling anthracnose of pomegranate.

Summary

6. SUMMARY

An investigation on management of leaf spot diseases of arecanut was carried out with reference to isolation, proving pathogenicity and symptomatology of the pathogens, cultural, morphological characters, molecular characterisation and physiological studies of the pathogens, survey on the incidence and intensity of leaf spot diseases, effect of weather factors on the incidence and intensity of the diseases, evaluation of fungicides, botanicals and bioagents against *Pestalotiopsis palmarum* and *Colletotrichum gloeosporioides* in the laboratory and evaluating the best fungicides, botanical, bioagent and their combinations on young areca seedlings and young areca palms in the field. The results obtained are summarized here under.

Three fungal isolates viz., '1', '2', '3'were isolated from Instructional Farm (I.F), Vellayani and Peringamala in Thiruvananthapam dist. and Balussery in Calicut dist. Artificial inoculation of the three fungal isolates was done on young areca seedlings. Isolate '1' produced symptoms comprising ashy brown centre surrounded by brown margin after eight days of inoculation, Isolate '2' produced brown spots with yellow halo surrounding it on the ninth day. Symptoms produced by isolate '3'were small brown coloured spots on the seventh day.

Based on the cultural and morphological characters, the isolates '1', '2', '3' were identified as *Pestalotiopsis palmarum*, *Colletotrichum gloeosporioides* and *Phomopsis palmicola*. Agarkar Research Institute identified these pathogens and deposited in the National Fungal Culture Collection and allotted *Pestalotiopsis* sp. with Accession No 3153. and *Colletotrichum gloeosporioides* with Accession Nos.3152 and 3154. Molecular characterisation of the pathogens collected from Balusserry, Calicut and Vellayani was done at the Rajiv Gandhi Centre for Biotechnology and identified the pathogens by the amplification of ITS region. Gel electrophoresis of the ITS region yielded a single fragment of approximately 650 bp in size.

Their identity was confirmed in the NCBI database and the sequences were submitted to GenBank *Pestalotiopsis palmarum* KF481949 and *Colletotrichum gloeosporioides* KF496900, KF496901,KF496902 and *Phomopsis palmicola* KF496903, KF496904, KF496905.

The phylogenetic tree analysis showed that the all the leaf spot pathogens grouped together distinctly. In the multiple alignment sequences, similarity was observed in the isolates obtained from same geographical regions than the isolates from different regions.

The leaf spots produced by *P. palmarum* were characterised by round to oval and brown to dark brown spots with characteristic brown bands along the margin, which were uniformly distributed all over the lamina. The arecanut plants infected by *C.gloeosporioides* showed small, circular or oblong to irregular brownish spots with or without a yellow halo.The symptoms of leaf spot caused by *Phomopsis. palmicola* were round to oval brownish spots confined to tips of the leaves.

On Potato Dextrose Agar (PDA), *P.palmarum* produced white cottony mycelium and black coloured fruiting bodies containing five celled conidia with coloured intermediate cells. Conidia measured 20.00 - 25.00 µm in length × 6.00 - 7.50 µm in width. The upper end of the conical cell was characterized by long slender, colourless appendages of 15 to 25 µm length. *C. gloeosporioides* produced dense, cottony, dirty white to greyish mycelium with the production of characteristic pinkish conidial mass. Conidia measured 12.50 – 15.00 in length × 3.70 – 5.00 µm in width. Growth of *P. palmicola* on PDA was white, thin to moderate, flat mycelium with even margin. The conidia were hyaline, elliptical with pointed ends measuring 6.25 – 7.50 µm in length × 1.50 – 2.50 µm in size.

Cultural studies conducted revealed that PDA, Czapek's agar, Richards' agar and Sabouraud's agar were best for the growth of *C. gloeosporioides*. The optimum range of pH for *C. gloeosporioides* and *P. palmarum* were 5.0 to 7.0. However, maximum growth of both the fungus was recorded at 5.0 pH.

Survey on incidence on leaf spot diseases revealed that I.F, Vellayani had 75 % disease incidence with 34.5 Per cent disease index (PDI) and in Balussery 85.0% and Peringamala 83.33%. The disease occurred all-round the year with PDI ranging from 20.13 to 33.21 for the months from April 2012 to March 2013 and maximum disease intensity (33.21%) was observed in November when weather recorded max. temperature (30.2°C), mini. temperature (23°C), R H (96.4 %) and Rainfall (111.1 mm). Highly significant negative correlation was found with mini.temperature and PDI .

Under *in vitro, Trichoderma harzianum* inhibited *P. palmarum* and *C. gloeosporioides* to an extent of 62.77 % and 51.04 % and the botanical Garlic at 6% inhibited the pathogens to 88.54 and 50.80 % respectively. Out of seven fungicides tested under *in vitro* against *P. palmarum* and *C. gloeosporioides,* Propiconazole (0.070,.0375 and 0.019%) and Carbendazim (0.2, 0.1 and 0.05%) completely inhibited the pathogens. Out of the five systemic fungicides tested for compatibility with *Trichoderma harzianum* , Garlic at 6% and Azoxystrobin at 0.1% were compatible with 81.21 and 72.09 per cent compatibility respectively.

Studies conducted on the management of leaf spot diseases on arecanut seedlings showed that out of nine treatments, foliar spray of Carbendazim at 0.1 per cent twice at 15 days interval was the most effective and was on par with Propiconazole at 0.075 per cent and Difenconazole at 0.1 per cent followed by Azoxystrobin 0.1 per cent +*T. harzianum* 2 per cent and they significantly reduced the leaf spot diseases on arecanut seedlings.

Studies conducted on the management of leaf spot diseases on young areca palms grown in I.F.Vellayani revealed that three sprays at 15 days intervals of Carbendazim at 0.1 % (50.15 reduction in PDI) which was the most effective and on par with Propiconazole at 0.075 % (48.61% reduction) followed by Azoxystrobin 0.1 %+*T. harzianum* 2 %(48.29 % reduction) were highly effective and reduced significantly the leaf spots of areca palms. Azoxystrobin 0.1 % + *Trichoderma harzianum* 2 % recorded 41.5% reduction in PDI .

The study revealed that the leaf spot in arecanut is caused by more than one fungus viz., Pestalotiopsis palmarum, Colletotrichum gloeosporioides and Phomopsis palmicola which is severe (33.21 PDI) when weather factors recorded maximum temperature (30.2°C), minimum temperature (23 °C), relative humidity (96.4%) and rainfall (111.1mm). The disease could be successfully managed *in vitro* by bio control agent Trichoderma harzianum, botanical garlic @ 6 % and fungicide Carbendazim (0.1%), Propiconazole (0.075%). In the *in vivo* conditions successful disease management can be done by spraying Carbendazim (0.1%), Propiconazole (0.075%) and Azoxystrobin (0.1%) + *T. harzianum* (2%).

Future line of work:

1. Survey on the leaf spot diseases of arecanut in the major areca growing areas of Kerala and their correlation with weather data.
2. Possible interrelationships between the leaf spot pathogens in causing the disease.
3. Standardization of spray schedule for controlling the leaf spot diseases.
4. Study of variability in the pathogens collected from major arecanut growing areas.

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*** Originals not seen**

APPENDIX -1

Composition of different media

1) Potato dextrose agar (PDA)

Potato peeled - 200 g

Dextrose-20 g

Agar-agar -20 g

Distilled water -1000 ml (volume to make up)

2) Oat meal agar (OMA)

Oat flakes -30 g

Agar-agar -20 g

Distilled water -1000 ml (volume to make up)

.3) Host extract agar (HEA)

Healthy greengram plant shoots- 200 g

Agar-agar -20 g

Distilled water- 1000 ml (volume to make up)

4) Czapek's agar

Sucrose ($C_6H_{12}O_6$) -30 g

Sodium nitrate ($NaNO_3$)- 20 g

Potassium dihydrogen phosphate (KH_2PO_4)- 1.0 g

Magnesium sulphate ($MgSO_4$-$2H_2O$)- 0.5 g

Potassium chloride (KCl)- 0.5 g

Ferrous sulphate ($Feso_4.7H_2O$) -0.01 g

Sucrose -30.0g

Agar-agar -20 g

Distilled water -1000 ml (volume to make up)

5) Malt extract agar

Malt extract -20 g

Agar-agar -20 g

Distilled water -1000 ml (volume to make up)

6) Sabouraud's agar

Dextrose- 40 g

Peptone -10 g

Agar-agar- 20 g

Distilled water -1000 ml (volume to make up)

7) Yeast extract agar

Yeast- 20 g

Agar-agar- 20 g

Distilled water-1000 ml (volume to make up)

8) Richard's agar

Sucrose -50 g

Potassium dihydrogen phosphate -5 g

Potassium nitrate -10 g

Magnesium sulphate -2.5 g

Ferric chloride- 0.02 g

Agar agar- 20 g

Distilled water- 1000 ml (volume to make up)

9) Potato carrot agar

Grated potato -20 g

Grated carrot -20 g

Agar agar -20 g

Distilled water- 1000 ml (volume to make up)

.10) Corn meal agar

Corn flakes- 60 g

Agar agar- 20 g

Distilled water- 1000 ml·(volume to make up)

11) Martin's rose Bengal agar

Dextrose : 10.0 g

Peptone : 5.0 g

KH_2PO_4 : 1.0g

$MgSO_4$. 7 H_2O : 0.50 g

Rose Bengal : 33mg/L

Streptomycin : 30.0g

Agar : 20.0g

Distilled water: 1000ml (volume to make up)

12) Nutrient Agar

Beef extract : 3.0 g

Peptone : 5.0 g

NaCl : 8.0g

Distilled water : 1000ml (volume to make up)

MANAGEMENT OF LEAF SPOT DISEASES OF ARECANUT (*Areca catechu* L.)

By

VIJAYARAJ. D

(2011 - 11 - 169)

ABSTRACT

of the thesis submitted in partial fulfilment of the requirement for the degree of

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ABSTRACT

The present investigation on **"Management of leaf spot diseases of arecanut (*Areca catechu* L.)"** was conducted at the Department of Plant Pathology, College of Agriculture, Vellayani, during 2011-2013. The objectives were to study the leaf spot diseases affecting young areca palms and to develop an integrated management practice to contain the diseases.

Three pathogens were obtained from the leaf spots. *Pestalotiopsis palmarum* Cooke. was isolated from dark brown or grey spots with brown bands and it produced white cottony mycelium with black coloured fruiting bodies on PDA. *Colletotrichum gloeosporioides* (Penz.) Penz and Sacc. was isolated from brown spots with yellow halo and also from grey spots without any margin and it produced dense, cottony, dirty white to greyish mycelium with pinkish conidial mass on PDA. *Phomopsis palmicola* was obtained from round to oval brownish spots, mycelium on the PDA was dull white, flat mycelial growth with even margin. All the cultures were molecularly characterized and identified as *P. palmarum*, *C. gloeosporioides* and *P. palmicola* based on the sequences obtained amplification of the ITS regions and diversity was observed in the sequences of the isolates from different geographical locations. Among the different pH levels, maximum radial growth of *P. palmarum* and *C. gloeosporioides* was observed at pH 5.0.

Disease occurred in all the locations surveyed and PDI of 34.5 was present in I.F. Vellayani. Incidence ranged from 75-85 per cent and the disease was restricted to 3-4 basal leaves only. Correlation coefficient between PDI and weather parameters showed that minimum temperature had significant negative correlation (-0.6725) with PDI. Five fungal and two bacterial organisms were isolated from phyllosphere and rhizosphere of healthy areca palms and one fungi and two bacteria were showing antagonistic activity against the pathogens.

In vitro evaluation - *T. harzianum* was effective in inhibiting the growth of *P. palmarum* (62.77%), *C. gloeosporioides* (51.04%). Out of the seven fungicides evaluated Propiconazole at (0.075, 0.037, 0.018%) and Carbendazim (0.2, 0.1, 0.05%) completely inhibited the growth of *P. palmarum* and *C. gloeosporioides.* Among five botanicals tested garlic at (2, 4 and 6%) was effective on *P. palmarum* and *C. gloeosporioides.* Garlic at 6% and azoxystrobin at 0.1% were compatible with *T.harzianum* upto 82.21% and 72.09% respectively. All other systemic fungicides tested were completely incompatible with the biocontrol agent.

An experiment was conducted with eight treatments and one control on seedlings in the green house. All the treatments tested were significantly effective against leaf spot diseases of arecanut. After 30 days of second spray the most effective treatment was Carbendazim with 50.15 % reduction in PDI, which was on par with Propiconazole which recorded 48.61 % and Difenoconazole with 48.29%.

For field level the experiment was conducted with eight treatments and one control on 4-7 yr. old young areca palms in the Instructional Farm, Vellayani. All the treatments tested were significantly effective against leaf spot diseases of arecanut. After 30 days of third spray the most effective treatment was Carbendazim with 39.33 % reduction in PDI which was on par with Propiconazole which recorded 38.90 % reduction. These two treatments were significantly superior over all other treatments, followed by combination treatment Azoxystrobin + *T.harzianum* which had 29.36 % reduction in PDI over control and pre- treatment.